THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XXX JANUARY–FEBRUARY, 1954 NUMBER 1

CHEMODECTOMA (NON-CHROMAFFINIC PARAGANGLIOMA) OF THE CAROTID BODY WITH DISTANT METASTASES

WITH ILLUSTRATIVE CASE *

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A distinctive neoplasm arising in the carotid body has long been recognized and many such tumors have been reported.[1,2] Now tumors arising in tissue embryologically and histologically similar to the chemoreceptor tissue of the carotid body are being recognized with increasing frequency. Most of these have involved the jugular or tympanic body in the region of the middle ear.[3] Because of the infrequency of distant metastasis of chemodectoma[4] in any location, its malignant potentialities have not been thoroughly appreciated. The following case is illustrative of the group in which metastasis has occurred.

Report of Case

Following a fall from a roof in July, 1940, a white man, then 50 years old, became aware of an asymptomatic, gradually enlarging mass in the left side of the neck. In early June, 1943, a mass less than 5 cm. in diameter in the left side of the neck below the angle of the mandible was explored surgically. The tumor was attached to, or located within, the carotid sheath and was interpreted grossly as a cancer. The surgeon stated that he was able to remove the major portion of the tumor. No microscopic examination of the excised tissue was made. On June 14, 1943, the patient was referred to the Colorado General Hospital by the surgeon with the request that the residual portion of the tumor be treated by x-ray irradiation.

Examination on admission showed a poorly defined, firm, residual mass, 2 to 3 cm. in diameter, below the angle of the mandible on the left. X-ray irradiation was delivered to the tumor between June 16 and June 29. The total dose was 3,600 r. (in air) using the following factors: 220 Kvp, 0.5 mm. Cu and 1 mm. Al filter, 50 cm. distance and 4 cm. portal. Thereafter the patient was seen in the Bonfils Tumor Clinic at irregular intervals. No appreciable change in the size of this mass was apparent during the subsequent several years. Some examiners expressed doubt that more than surgical and irradiation effects existed.

On June 19, 1947, he reported hoarseness for the preceding year. Examination

* Received for publication, May 11, 1953.

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showed paralysis of the left vocal cord. On June 9, 1949, left miosis and ptosis were noted and the left cervical mass was described as firm, 3 cm. in diameter, and without distinct borders. On June 25, biopsy of the firm tissue beneath the skin showed only scar tissue. In December, 1949, he complained of constant low back pain for several months. On June 13, 1950, enlargement of the left cervical mass to a diameter of 5 cm. was noted. The upper portion of the sternocleidomastoid muscle was adherent to the underlying mass and to the adjacent skin. Biopsy was repeated and showed a tumor of carotid body type.

Removal of the tumor by radical dissection was performed on July 5, 1950. The dissection extended anteriorly to the midline, posteriorly to the transverse processes of the cervical vertebrae, superiorly to the base of the skull, and inferiorly to the clavicle. Included in the tissues removed were the left carotid artery, external and internal jugular veins, vagus nerve, a portion of the left lobe of the thyroid gland, the sternocleidomastoid muscle, and a large segment of skin overlying the mass. The firm tumor was centered about the carotid bifurcation, extended into adjacent tissues, and was without clear borders. Study of the surgical specimen showed two large veins filled by tumor. Microscopic examination of these areas showed focal perforation of the wall of the vein by neoplasm, which extended as a bulbous mass into the lumen. Large masses of neoplasm were lying free within the lumen. Tumor surrounded the common carotid artery and extended into the adventitia, but did not involve media or intima. The infiltrative primary tumor consisted of large islands of cells separated by wide areas of dense fibrous connective tissue. A detailed description of the cytologic features will be given under Necropsy Findings.

Following the operation, the patient had persistent difficulty in swallowing all foods. During attempts at feeding, aspiration into the larynx was frequent. He received most of his food through a Levin tube. The probable cause of dysphagia was anesthesia of the hypopharynx due to sacrifice of the glossopharyngeal and vagus nerves.

On February 15, 1951, the patient complained of poorly localized, constant pain in the anterolateral portion of the left thoracic region. In October, 1951, frequent and persistent cough appeared. On May 15, 1952, he was awakened by cough and hemoptysis. He was admitted to the Colorado General Hospital with continued hemoptysis and died 5 hours later.

Thoracic Radiographs. An osteolytic lesion in the anterolateral portion of the left third rib with adjacent soft tissue density first became apparent in a chest plate on January 26, 1951. The soft tissue density was larger in later chest plates. Between January 26, 1951, and May 9, 1952 (Fig. 1), roentgenograms of the chest showed an increasing number and prominence of round densities near the hila of the lungs and an increasing upper mediastinal shadow.

Spinal Radiographs. Roentgenographic examination of the lumbar spine on December 16, 1949, showed slight coarsening of trabeculae combined with mottled rarefaction of the entire body of the fourth lumbar vertebra. On February 27, 1951, a radiograph showed increased prominence of this lesion (Fig. 2). In 1945 this vertebra was normal in a radiograph of the pelvis.

Necropsy Findings

At necropsy, a firm, gray-white sheet of tumor, averaging 2 cm. in thickness, covered the anterior and lateral portions of the trachea in the neck and was intimately applied to the remaining thyroid gland. The neoplastic tissue extended into the upper mediastinum where it

was more abundant and had a lobulated appearance, ended proximal to the bifurcation of the trachea, and slightly overhung the aortic arch. The trachea and bronchi were filled with partly clotted blood. Within the medial portions of all lobes of the lungs were firm, gray-white nodules up to 4 cm. in diameter, some encircling secondary bronchi and narrowing their lumen. One involved bronchus in the right lower lobe showed irregularly elevated mucosa with a gray-brown area of roughening, 5 mm. in diameter, interpreted as the probable site of the fatal intrabronchial hemorrhage. A similar nodule, 3.5 cm. in diameter, was found in the anterior wall of the right atrium of the heart, about one third bulging into the atrial cavity, the remainder within the pericardial sac. The anterolateral portion of the left third rib showed a fusiform thickening 4 cm. long. Cut section showed gray-white tissue filling and expanding the marrow spaces and extending into adjacent soft tissue. The cortex in this area was destroyed. The fourth lumbar vertebra was not examined through oversight. Several lower thoracic and upper lumbar vertebral bodies showed no gross tumor. No abnormality was found within the cranium.

Microscopic Examination (*Figs. 3 to 8*). The histologic appearances of the neoplastic tissue in the surgical specimen obtained on July 5, 1950, and in that obtained at necropsy were similar. The tumor was composed of polyhedral cells showing little variation in size and scattered single giant cells containing disproportionately large, frequently hyperchromatic, nuclei. Occasional cells contained two or more nuclei. Few mitotic figures were present. The cytoplasm was faintly outlined, lightly eosinophilic, and vacuolated. The nuclei were large, slightly eccentric, oval to round, well outlined, and contained a delicate chromatin net marked by numerous small granules. Pale to dark, small nucleoli were often seen. These cells were grouped without apparent pattern in round to elongated, often communicating compartments, outlined and separated by septa consisting of collagen and reticulum fibers, few fibrocytes, and numerous blood sinusoids, which varied widely in number and size. In some areas the vascular component predominated to the extent that narrow cords of tumor cells intervened between very vascular septa. Retraction of the cell groups from the septa was common. The tumor in the cervical and upper mediastinal areas differed from the other neoplastic masses by the presence of an extensive stroma of dense fibrous connective tissue. Large and small groups and single masses of tumor cells were widely separated by a stroma of interlacing bundles of dense fibrous connective tissue. Sec-

tions of two of the vertebral bodies showed microscopic foci of similar neoplasm. Within the metastatic lesions of the lungs, two small vessels, probably veins, contained tumor within thickened intima and the neoplasm bulged into the lumen. Several thin-walled small veins at the periphery of the metastatic tumors contained groups of neoplastic cells. The tissue sections from the radical neck dissection and from the necropsy were reviewed by Dr. Frank W. Foote, Jr., of Memorial Center, New York City, who concurred with the histologic diagnosis of non-chromaffinic paraganglioma.

Review of Literature

Cases of Chemodectoma with Metastasis to Regional Lymph Nodes

Seven cases of chemodectoma with neoplastic involvement of regional lymph nodes have been found in the literature.

Kopfstein[5] reported the case of a 34-year-old man who had had a left cervical tumor for 4 years. Examination of the surgical specimen revealed a tumor of the carotid body and a lymph node at its lower pole which was largely replaced by histologically similar tumor.

A tumor of the right carotid body present for 2 months in a 15-year-old boy was recorded by Shawan and Owen.[6] Removal of the tumor from the bifurcation of the right carotid artery was combined with excision of regional cervical lymph nodes. The pathologic diagnosis was "perithelioma of the carotid body with infiltration of the lymph nodes."

MacComb[7] reported 2 cases of chemodectoma with tumor in regional lymph nodes. A 24-year-old man had a tumor of the left carotid body for 2 years. The tumor was removed together with a segment of the left common carotid artery. A second operation was performed 20 months later because of local recurrence. Study of this surgical specimen revealed "recurrent carotid body tumor, with vein and node invasion." Many large lymph nodes were palpable on both sides of the neck 6 months later. He died 1 year after the second operation. Necropsy was not done. A 20-year-old woman had a tumor of the right carotid body for 18 months. Examination of the surgically removed neoplasm revealed a tumor of the carotid body and a "lymph node, showing metastasis or extension."

Winship, Klopp, and Jenkins[8] reported a neoplasm in the region of the left middle ear of a woman who had clinical signs indicating its presence at the age of 37 years. Seventeen years later, several firm lymph nodes up to 3 cm. in diameter were noted in the left cervical

region and tumor was present within the left external auditory canal. One enlarged lymph node removed surgically revealed almost complete replacement by chemodectoma. Surgical manipulation of the tumor within the external auditory canal provoked such severe bleeding that a specimen was not obtained from the primary tumor for biopsy.

Lahey and Warren[9] recorded a case in which a microscopically examined tumor of the glomus jugulare showed metastasis to one of five cervical lymph nodes excised. No other data were presented.

LeCompte[10] referred to a case of carotid body tumor with an adjacent lymph node involved by histologically similar neoplasm. No other data were presented.

An additional chemodectoma of a carotid body with cervical lymph node metastasis was contributed by Dr. S. K. Kurland of Denver and was studied in this laboratory. A 35-year-old woman had an asymptomatic cervical mass for 16 years. The tumor, which measured 33 mm. in diameter, was removed from the bifurcation of one common carotid artery. An adjacent lymph node, 13 mm. in diameter, contained an oval, subcapsular nodule, 3 mm. in diameter, microscopically similar to the primary tumor. A single mitotic figure was present in the neoplastic nodule within the lymph node; none was found in the primary tumor.

Cases of Chemodectoma with Distant Metastases

Eight cases of chemodectoma with distant metastases have been found in the literature. Sapegno[11] recorded the case of a 64-year-old woman who was hospitalized because of cough and hemoptysis and later died of bronchopneumonia. Necropsy showed a tumor of the right carotid body with metastasis to the right cervical lymph nodes, mediastinal lymph nodes, and the right lung with erosion of bronchi and extension into their lumen.

Bérard and Dunet[12] reported the case of a woman with a tumor of the right carotid body present for 3 years. When the tumor was removed surgically, the external carotid artery was ligated. Study of the surgical specimen showed that the tumor had penetrated the wall of an adjacent large vein and extended into the lumen as a polypoid mass. Left hemiplegia developed within 48 hours following operation and the patient died soon thereafter. At necropsy, a small metastatic nodule was found in the right lung.

Goodof and Lischer[13] recorded the case of a man who had had an

untreated tumor of the left carotid body for 20 years. Death at the age of 62 years was due to heart failure caused by syphilitic aortitis. At necropsy, a nodule, 15 mm. in diameter and histologically similar to the cervical tumor, was found within the pancreas.

Martelli[14] reported on a 44-year-old man who had had a right cervical mass for 3 years. Necropsy revealed a locally infiltrative tumor of the right carotid body and metastatic nodules in lungs and liver.

A widely metastasized chemodectoma in a man who died at the age of 27 years was reported by Pendergrass and Kirsh.[15] The neoplasm was initially interpreted as angiosarcoma primary in the left supraorbital region. X-ray irradiation was directed to that area 2½ years and 1 year before death, without significant change in size of the massive tumor. Four months before death, fracture of the right femur occurred at the site of an osteolytic lesion. Necropsy revealed a large tumor in the entire left orbital region, a smaller tumor in the left side of the neck below the angle of the jaw, and nodules of tumor in the left parietal bone, lungs, liver, sternum, ribs, vertebrae, and pelvic bones. On subsequent review of the case, the neoplasm was classified as a primary tumor of the left carotid body with metastases to the left orbit and other sites listed.

Donald and Crile[16] reported upon a man who had had a tumor of the left carotid body removed when he was 27 years old. Fourteen years later paraplegia occurred and laminectomy revealed tumor extending from the fifth and sixth thoracic vertebrae and compressing the spinal cord. The accessible portion of this tumor was excised. This operative site was again explored 11 years later when neurologic symptoms reappeared and a recurrence of a similar neoplasm was found. Roentgenograms revealed lesions in three ribs, the sternum, and right ilium. The patient died 2 months after the last operation. Necropsy was not done.

A tumor in the region of the left middle ear arising in the jugular body or the tympanic body was reported by Lattes and Waltner.[3] The woman died at the age of 71 years, 20 months after the appearance of the tumor in the left external auditory meatus. At necropsy, there was extensive local invasion by the primary neoplasm and one large metastasis was found in the liver.

Spotnitz[17] recorded the case of a man with an untreated cervical tumor for 19 years. For 9 months prior to death at the age of 83 years, an ulcerated, enlarging tumor was present in the right thoracic region. Necropsy revealed a tumor of the left carotid body with extension into adjacent tissues and metastasis to thyroid gland, cervical

lymph nodes, chest wall, kidneys, pancreas, and interventricular septum of the heart.

Discussion

The seven cases[5-10] reported in the literature and the one examined in this laboratory and briefly described in this communication have been accepted as valid instances of chemodectoma which metastasized to regional lymph nodes.

The eight cases[3,11-17] reported in the literature and the one described in detail from this laboratory have been accepted as genuine examples of chemodectoma which metastasized to sites beyond regional lymph nodes. Goodof and Lischer[13] interpreted the small tumor within the pancreas and the tumor of the carotid body as an example of multicentric origin of a similar neoplasm within chromaffin tissue. Since the time of that report the identity of the chemoreceptor system has been established,[18-20] and such tissue is unknown in the region of the pancreas. The intimate relationship of the neoplasm to the pancreas, exhibited in this case, suggests a metastatic tumor.

In each of the recorded cases of chemodectoma with distant metastases, the primary neoplasm and one or more of the metastases were examined microscopically. In only one case[3] was photomicrographic proof of metastasis given. In the others[11-17] sufficient description allowed acceptance as true examples of chemodectoma with distant metastases.

The histologic features of the chemodectoma with distant metastases and of the tumor showing lymph node involvement, as described in this paper, were compared with those of five other tumors of the carotid body on file in this laboratory. These five were removed surgically and none showed evidence of local infiltration or metastasis. The two cases with metastases showed no unique features which could serve to distinguish them from the five non-metastasizing tumors, except for the presence of infrequent mitotic figures in both metastasizing tumors and the invasion of veins in one. The latter feature was occasionally simulated in the clinically benign group by retraction of a group of tumor cells from an adjacent connective tissue trabecula together with spillage of erythrocytes from the blood vessels into this space, which gave a superficial resemblance to neoplastic tissue within a blood vessel. The size of the groups of tumor cells, the size and nuclear-cytoplasmic ratio of the tumor cells, and the presence of giant cells did not distinguish the malignant from the non-metastasizing group. The giant cells were largest, most numerous, and most bizarre in one of the clinically benign tumors. In this specimen, the

tumor cells contained the largest and darkest nucleoli but had the smallest nuclear-cytoplasmic ratio.

The infrequent and questionably significant positive differences found in this very small group of neoplasms suggest that histologic differentiation of biopsy material into benign and malignant groups is unwarranted at the present time.

Summary

A case of chemodectoma of the carotid body with multiple distant metastases and one with neoplastic involvement of an adjacent lymph node have been recorded. Also, seven reported cases of chemodectoma with involvement of regional lymph nodes and eight with distant metastases have been reviewed. Comparison of the two malignant chemodectomas with five which were benign revealed no apparent significant histologic differences.

Assistance in preparing this paper was obtained from Dr. R. M. Mulligan, Professor of Pathology, University of Colorado School of Medicine.

ADDENDUM

Since this paper was submitted for publication, Morfit, Swan, and Taylor have proposed a method of treatment of carotid body tumors. In their communication,[21] the cases recorded in this paper were utilized as their cases 1 and 4.

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[*Illustrations follow*]

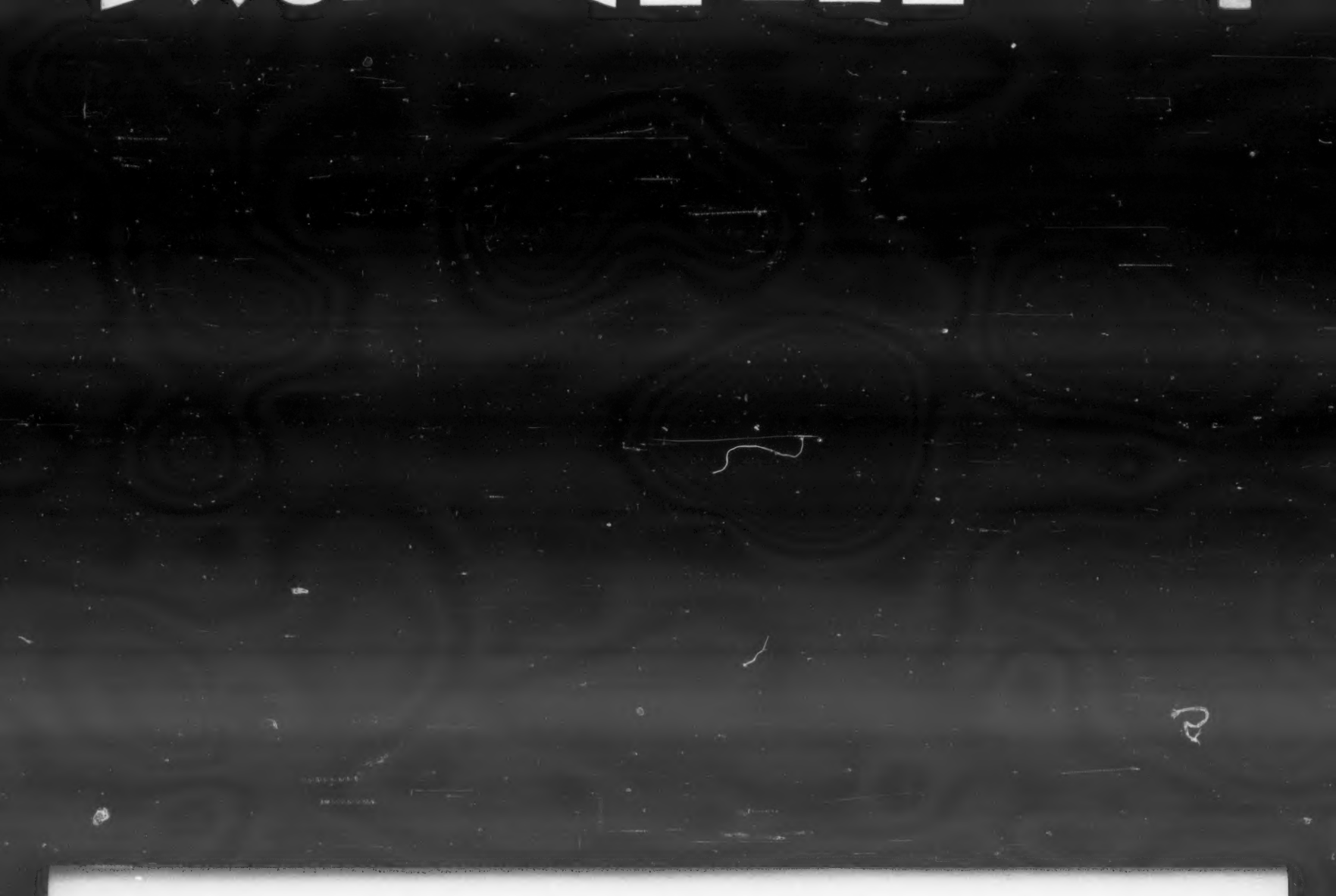
LEGENDS FOR FIGURES

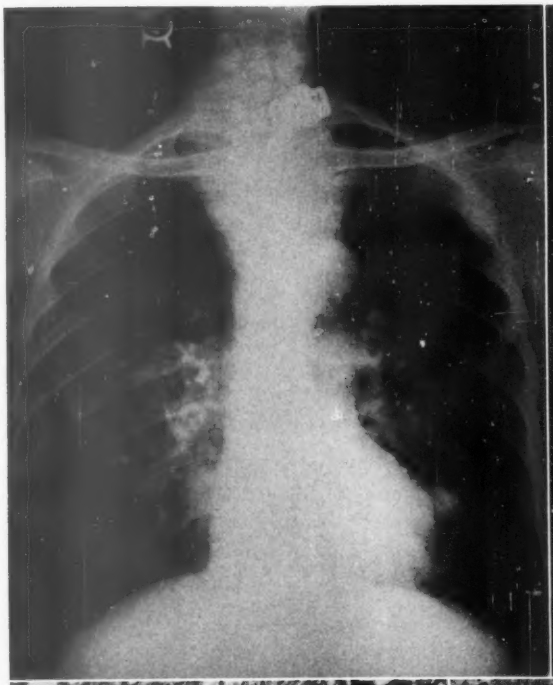
FIG. 1. Radiograph of chest on May 9, 1952, demonstrating masses in lungs and upper mediastinum and soft tissue density adjacent to the left third rib. × 1/15.

FIG. 2. Radiograph of lumbar vertebrae on February 27, 1951, demonstrating destruction of the fourth lumbar vertebra. The second lumbar vertebra is at the top of the figure. × 2/15.

FIG. 3. Tumor in the surgical specimen obtained on July 5, 1950. Masses and single groups of tumor cells are separated by a large amount of fibrous connective tissue. Hematoxylin and eosin stain. × 90.

FIG. 4. Tumor in the wall of the right atrium of the heart. At the left is myocardium. Hematoxylin and eosin stain. × 90.





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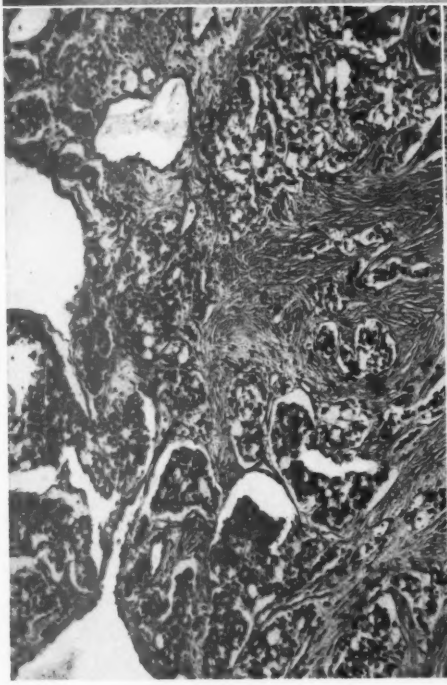

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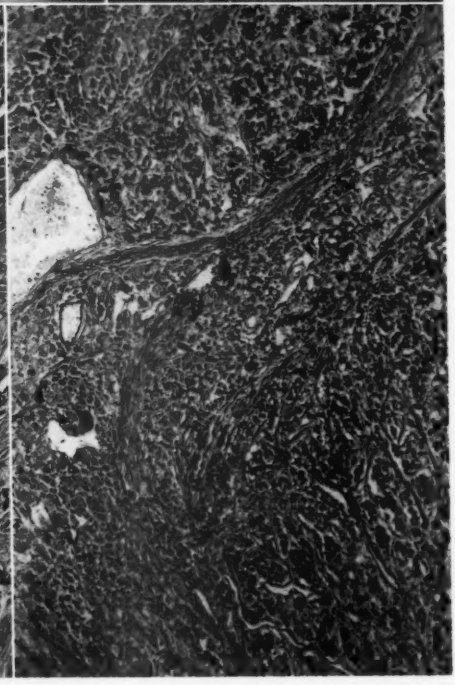

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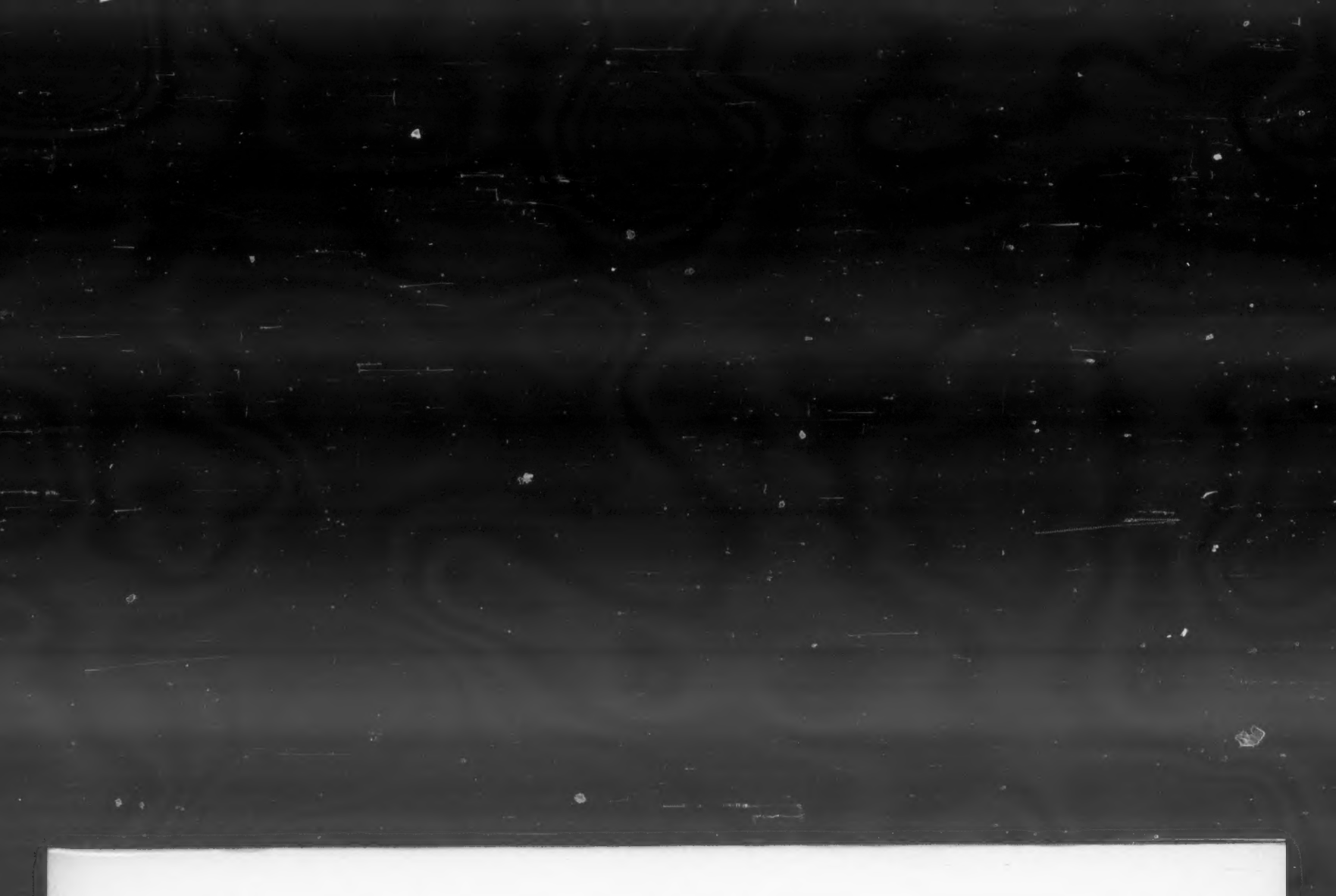
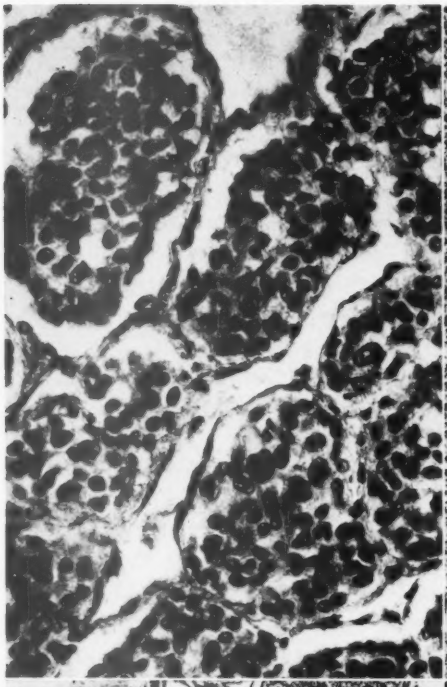
FIG. 5. Tumor in the wall of the right atrium of the heart showing typical pattern. A blood sinusoid crosses the center. All groups of tumor cells show retraction from the fibrous connective tissue septa. Hematoxylin and eosin stain. × 350.

FIG. 6. Tumor in lung surrounding and destroying bronchial cartilage and extending into bronchial mucosa. Hematoxylin and eosin stain. × 50.

FIG. 7. Edge of tumor in lung. A portion of a bronchus is in the left upper corner. Wilder's reticulum stain. × 40.

FIG. 8. A small blood vessel in the lung surrounded by and containing tumor. Hematoxylin and eosin stain. × 200.





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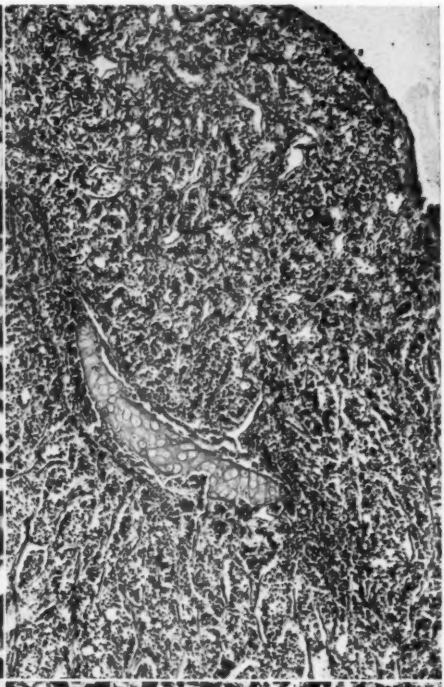

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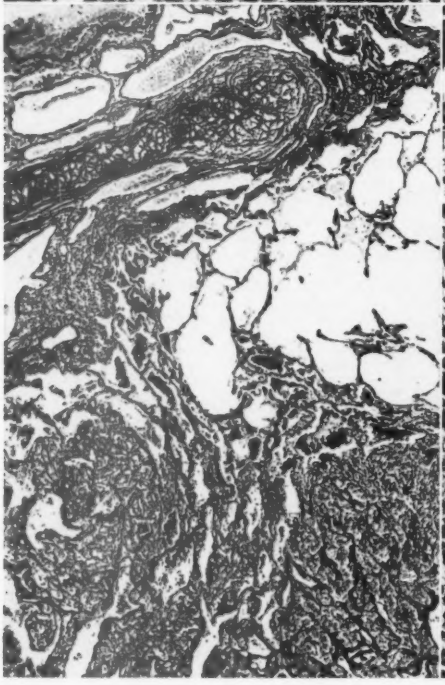

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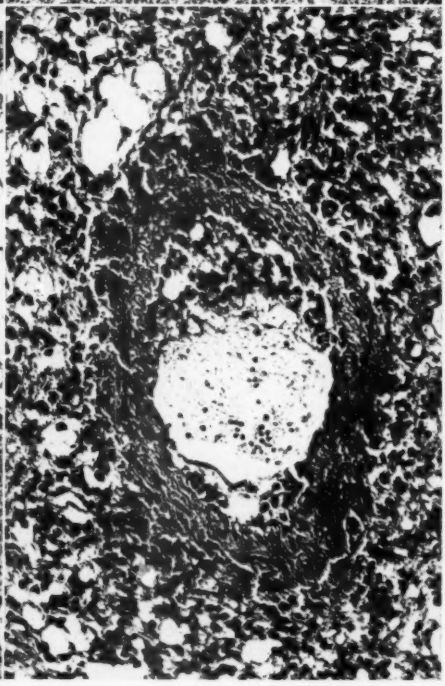

8

PREINVASIVE CARCINOMA AND PRECANCEROUS METAPLASIA OF THE CERVIX

A Serial Block Survey *

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This study of the uterine cervix is a portion of an investigation of the early manifestations of neoplasia in various organs. Observations on preinvasive carcinoma (carcinoma *in situ*) in several viscera have given solid support to the concept that this lesion is an initial phase of invasive epithelial neoplasia and that in one region it often arises in multiple foci.[1,2] With respect to the cervix, however, there has arisen uncertainty as to the identity of similar lesions. Doubt has been expressed that preinvasive carcinoma occurs frequently in the uterus,[3] that the criteria by which it may be identified are valid, or that it represents a process which must inevitably become invasive.[4,5] The present study adds to the large body of data accumulated in support of the identity and malignant neoplastic nature of this condition.[6-11]

We have defined preinvasive carcinoma as an alteration of growth and differentiation of surface epithelium, simulating that observed in a malignant neoplasm but differing only in the absence of evidence of invasion. In the cervix it is necessary, and usually possible, to distinguish this lesion from such conditions as squamous metaplasia, leukoplakia, inflammatory distortion, condyloma, and a pertinent process which we have chosen to classify as precancerous metaplasia.

Material and Methods

Three groups of specimens have been investigated.†

Group A. Prior to 1946 the diagnosis of preinvasive carcinoma of the cervix had not been entertained at the Cincinnati General Hospital. Accordingly, all available cervical biopsy material accumulated prior to that date was reinvestigated and those sections listed as chronic cervicitis or squamous metaplasia were reviewed. There were 718 such specimens. For those in which review led to a diagnosis of

* This investigation was supported in part by a research grant from the National Cancer Institute, National Institutes of Health, Public Health Service, and in part by the John R. Stark Memorial Fund.

Presented at the Fiftieth Annual Meeting of the American Association of Pathologists and Bacteriologists, St. Louis, April 2, 1953.

Received for publication, June 17, 1953.

† This study is in continuation and completion of a survey initiated by Drs. Fred B. Aurin, Samuel C. Capps, Daniel F. Richfield, and Norman Zheutlin.

preinvasive carcinoma, the subsequent courses of the patients we investigated.

Group B. Between 1946 and 1951 there were 18 instances in which a diagnosis of preinvasive carcinoma was made and followed by total hysterectomy. In addition the uteri of 11 women removed for other causes were unexpectedly found to contain preinvasive carcinoma. From each of these 29 specimens full length four-quadrant blocks of the cervix were prepared for histologic study. The blocks included most or all of the endocervical canal, the external os, and as much of the vaginal surface as could be included. Studies of this material served to locate the distribution of the lesion in relation to the cervical canal.

Group C. During the past 2 years 14 uteri have been removed following a biopsy diagnosis of preinvasive cervical carcinoma. An additional uterus was resected following a diagnosis of precancerous metaplasia. These uteri were opened by a single incision, the specimen flattened, fixed, and serial radial blocks of the entire cervix prepared.[12] The number of blocks varied from twelve to fifteen per uterus and included the full length of the cervical canal, the lower uterine segment, and the attached cuff of vagina. From each block large paraffin sections were prepared at four or five levels.[13] Thus a histologic survey of some forty to fifty sections from each cervix permitted accurate localization of pertinent lesions. A diagrammatic sketch was prepared of the opened gross specimen. Upon this were indicated the areas of block preparation and the extent and distribution of the lesions encountered (Fig. 1). It was thus possible to construct a panoramic map of the pathologic changes observed.

Histologic Criteria

Squamous Metaplasia. Non-keratinizing stratified squamous epithelium within the endocervical canal presents the essential nature of squamous metaplasia.[12] The cells are well differentiated, regularly oriented, and, above the basal columnar layer, polyhedral in configuration. The nuclei are spherical and, though moderately varied in size, are relatively uniform in shape and polarity. Save in the basal columnar layer, mitotic figures are quite unusual and never bizarre. Invading leukocytes may mimic mitotic figures but are readily distinguished from them. Distortion attendant upon inflammation may simulate neoplastic atypism, but this can be easily resolved by careful scrutiny with the high-power lens (Fig. 2).

Metaplasia within endocervical glands is a common concomitant.

Squamous elements characteristically insinuate themselves between the gland epithelium and basement membrane and often proceed to complete replacement of mucus-secreting cells, filling the acini with clusters of uniform squamous epithelium.

Individual Cell Atypism. Individual cell atypism is considered a variant of precancerous metaplasia. Appearing within foci of squamous metaplasia or in the otherwise normal squamous epithelium covering the portio, the process is characterized by scattered and isolated cells in which striking atypism has developed (Fig. 3). The cells are large, misshapen, with bizarre often lobulated nuclei and irregularly distributed chromatin. They are not related to the basal columnar layer and may exhibit either mitotic or amitotic division. Polarity is lacking, a feature which contrasts sharply with the surrounding epithelium.

Precancerous Metaplasia. Characteristically, the alteration in precancerous metaplasia is limited to the deeper two thirds or less of the stratified mucosal epithelium, the superficial layers exhibiting rather abrupt transition to a normal appearing squamous character (Figs. 4 and 5). The more deeply lying cells, however, are pleomorphic, often with abundant cytoplasm, and display a loss of orientation. There is variation in nuclear size, chromatin content, and polarity. Mitotic figures are common and may be multipolar. A similar change occurs within glands and may be difficult to differentiate from preinvasive carcinoma. In the metaplastic process, the cells are usually polyhedral and the nuclei more often vesicular.

Of interest is the line of separation between the superficial and deeper layers which on lower power examination frequently proves to be oblique but produces a much longer (more acute) angle than in preinvasive carcinoma.

Preinvasive Carcinoma. In preinvasive carcinoma the entire depth of the mucosal epithelium is the seat of profound alteration, with the superficial epithelium as disorderly as its deeper counterparts.[9,11,12,14,15] At the line of juncture with non-neoplastic squamous epithelium, one may often observe an oblique line of separation which is much shorter and less acute than that noted in precancerous metaplasia. Extension into glands follows the pattern described for squamous metaplasia. The acinar basement membrane remains intact but with complete filling it may be difficult to determine whether or not this has been breeched.

The epithelium in this lesion tends to be less pleomorphic and polyhedral and proves to be more uniformly spindle shaped (Figs. 6 and 7). Although arranged perpendicularly to the basement mem-

brane, there is no regular polarity. Cytoplasm is scanty and basophilic. Nuclei are ovoid, elongated, hyperchromatic, and mitotic figures are common. Bizarre mitotic figures are frequently observed and may occur at any level.

Early Invasive Carcinoma. In addition to the features described under preinvasive carcinoma, in early invasive carcinoma there is disruption of the basement membrane with either perceptible invasion of superficial stroma or actual entrance into subjacent lymphatic channels.[3,9,11] It may be exceedingly difficult to establish the fact of invasion in tissue for biopsy which is the seat of an intense inflammatory exudate.

Observations

Group A. Reinvestigation of 718 cervical biopsy sections from group A, not previously considered the seat of preinvasive carcinoma, resulted in the recognition of this lesion in 13 instances. This should not be taken to represent the frequency of occurrence during this period. Many examples were undoubtedly included among the cases classified as squamous cell carcinoma and this group was not reviewed. Attempts to follow up the 13 patients were rewarded in 9 cases, the remaining 4 being lost to observation. Among the 9, 5 were found to have an invasive cervical neoplasm within a short period after the initial biopsy. These were obviously instances in which the biopsy provided tissue adjacent to but not within the region of existing invasion. Three others were found to have frankly invasive cervical cancer at intervals of 2, 6, and 12 years, respectively. One patient was observed 10 years after biopsy and was said to have had no clinical evidence of carcinoma. A second biopsy was not made and she has not been seen for 7 years. Thus only one of the 13 patients is known to be free of cervical neoplasm after adequate follow-up.

Group B. Four-quadrant sections were made from cervical segments of 29 uteri in group B. In 19 of these, preinvasive carcinoma was evident in two or more quadrants. In 7, the lesion appeared in a single focus only. Three of the resected uteri failed to show the preinvasive neoplasm, despite its confirmation in the original biopsy sections. These patients had received neither external radiation nor radium preoperatively.

Although the examination of quadrant blocks is a fair method of determining the distribution of lesions, it may not be considered conclusive. Nonetheless, the extent of the lesions observed by this limited means was considerable. In at least 19 cases, the lesions constituted either broad plaques or consisted of several independent foci.

It is of interest that in this group of specimens precancerous metaplasia also was present in 25 of the 29 uteri.

Group C. Among the 14 uteri subjected to serial block survey in group C, two or more independent foci of preinvasive carcinoma were demonstrated in 7. In 4 cases the lesions as reconstructed were found to be superficial plaques. Two encompassed one half or more of the cervical circumference and 2 completely encircled the entire canal. In 3 of the 4 uteri with broad plaques, there were also multiple, independent, neoplastic foci. In 3 specimens only a single small focus was found, although in 2 of these the preinvasive lesion had been observed in two of the four-quadrant specimens secured preoperatively for biopsy.

In 13 of these 14 specimens the lesion of precancerous metaplasia accompanied preinvasive carcinoma (Fig. 1).

Discussion

The lesion of preinvasive carcinoma represents a well defined entity, the histologic structure of which differs from that common to non-neoplastic states in the cervical region. Indeed, the pattern is such that distinction from acceptable neoplasm is made only by the absence of invasion of supporting tissues. Surveys by serial and quadrant blocks indicate that the lesion usually involves much of the circumference of the canal. Often the process obviously has multiple sites of origin, thus paralleling observations made upon neoplasms in other viscera.[1,2,16] It is of interest that Rubin,[17] reporting observations on "incipient" cervical carcinoma in 1910, considered one of his two cases to be of multicentric nature. Similar experiences have been recorded by others.[8,9,12,18]

Whether the site of origin is in the portio vaginalis or within the endocervical canal has been a matter of disagreement.[7,9,12,14,18,19] In the present group of cases preinvasive carcinoma originated within the canal in every instance and extended to the portio in only 2 uteri. There were 4 specimens, however, in which precancerous metaplasia appeared within the mucosa of the portio.

Although there remains little question among competent authorities as to the existence of preinvasive carcinoma, there is doubt in some quarters concerning its inevitability of progression. So sharp is this point of disagreement that one is led to suspect that a single lesion is not at issue. A critical review of the illustrations reproduced in many of the publications pertaining to this subject compels appreciation that not all of the lesions labeled carcinoma *in situ* are similar. It is quite

possible that a recognition of the distinctions between preinvasive carcinoma and precancerous metaplasia may aid in the clarification of this problem. The precancerous process often manifests disturbing cytologic features and may well be classified by some, we believe unjustifiably, as a neoplastic process.

The term precancerous metaplasia is admittedly not altogether a satisfactory one. We have chosen it, however, in preference to other designations such as "basal cell hyperplasia,"[5,14] "anaplasia,"[11] "dysplasia,"[20] and "atypical hyperplasia" or "atypical squamous metaplasia"[12] which appear to be even less suitable. They are expressions which denote a histologic variation, indicating uncertainty in the mind of the pathologist, but they give little or no cause for concern to the clinician. Experience, however, points to the need for some designation of the lesion's dangerous potentialities. The term precancerous metaplasia has such connotations. As with precancerous lesions elsewhere in the body, it commonly accompanies malignant neoplasm, it often presages its development, but it does not inevitably lead to carcinoma.[11,14,19-21]

Among the 44 specimens comprising groups B and C, precancerous metaplasia accompanied preinvasive carcinoma in 39 (89 per cent). In a cursory survey of a large volume of material with invasive carcinoma of the cervix, precancerous metaplasia was almost always detectable in the mucosa adjacent to the neoplasm.

Follow-up data in patients in whom only precancerous metaplasia has been encountered in biopsy material are limited, but they are of significance. There have been 31 cases in one recent series. To 8 of these patients no treatment was administered and follow-up (6 months to 5 years) has shown no serious sequelae. In 17 the uterus was resected at various intervals without preliminary radiation. Nine specimens contained a residuum of the lesion and in 5 it was not found in search sections. In 3 cases preinvasive carcinoma was encountered. Five patients had radium insertion followed by hysterectomy. The cervical mucosa was destroyed and none was apparent in the resected specimens. An additional patient, cited subsequently, showed only precancerous metaplasia in sections from the resected uterus but suffered recurrent carcinoma in the vaginal vault 1½ years later.

Thus it would appear that a lesion which is so often found in association with one or another phase of malignant neoplasia, and which, in a limited number of cases when present alone, may ultimately result in carcinoma, deserves more than a cytologically descriptive designation.

Finally, our experience in respect to the concomitance of preinvasive carcinoma in biopsy specimens with invasive neoplasm elsewhere in the cervix justifies comment. This, of course, has been remarked by many others.[9-11,14,22]

The association is well illustrated by the 5 cases in group A in which frank invasion was recognized within a few weeks of biopsy studies which failed to demonstrate this feature. It is of further interest that among the 44 specimens comprising groups B and C unsuspected invasive carcinoma was identified in one or more of the cervical blocks in 8 uteri (18 per cent). A biopsy diagnosis of preinvasive carcinoma does not exclude the possibility of an invasive lesion.

Even meticulous search for invasive carcinoma following hysterectomy for preinvasive carcinoma may not conclusively eliminate this possibility.[22,23] This is emphasized by our experience with the following cases.

A patient (A. N., age 32, no. 109449) in whom preinvasive carcinoma was detected by biopsy at 4½ months' gestation was treated by prompt total hysterectomy (Fig. 8). No evidence of invasion appeared in quadrant blocks of the cervix although precancerous metaplasia was observed in the mucosa of the portio. Two years later a nodule of squamous cell carcinoma appeared at the vaginal apex (Fig. 9).

Another patient (A. B., age 48, no. 203068) in whom only precancerous metaplasia was demonstrated (extending onto the portio) developed a neoplastic vaginal lesion 1½ years after total hysterectomy.

In a third patient (C. T., age 36, no. 43692) preinvasive carcinoma appeared in four-quadrant biopsy specimens during the sixth month of pregnancy (Fig. 10). Gestation was permitted to continue to term after a second biopsy revealed a similar process. Shortly after delivery, hemorrhage necessitated hysterectomy. The surgical specimen revealed endocervical carcinoma which had invaded beyond the anatomical limits of the uterus (Fig. 11).

Post-partal regression of cervical epithelial atypism noted during pregnancy has led to caution in the interpretation of these changes.[11,20,24-27] It has been inferred that a diagnosis of preinvasive carcinoma rendered on tissue removed during pregnancy may be of doubtful validity. The recommendation that such patients be treated conservatively and that the status of the cervix be re-evaluated by smear or biopsy during the post-partal state[28] has received rather wide accord. However, Greene and his co-workers[29] carried out just such a study and found that lesions of this nature were disturbingly persistent well into the post-partum period. It was concluded that if the criteria

for preinvasive carcinoma were met in tissue procured prior to delivery, the lesion should not be expected to regress. Two of our cited cases are pointedly apropos. Whether or not the same conclusions may apply to precancerous metaplasia requires further investigation, in the course of which a clear differentiation between the two types of lesion must be maintained.

If the two conditions are distinguishable, as we believe they are, then it is possible that the recorded instances of reversibility may represent precancerous metaplasia and not preinvasive carcinoma. A study of this character would require experience, strict observance of cytologic detail, and critical histologic judgment. Requirements of this order are not peculiar to interpretation of diseases of the cervix.

Summary

Criteria are established for the histologic distinction between preinvasive carcinoma of the cervix and a lesion termed precancerous metaplasia.

The biologic characteristics of these lesions have been studied by several methods including large serial sections of resected uteri.

Both lesions occur in multiple sites or as broad superficial plaques and it is unusual for either to appear at a significant distance external to the squamo-columnar juncture.

Precancerous metaplasia accompanied preinvasive carcinoma in 89 per cent of 44 instances of the latter but was also encountered in its absence.

Unsuspected foci of invasion were detected in 18 per cent of the cases with preinvasive carcinoma.

Evidence indicates that invasion ultimately supervenes in, or actually accompanies, the majority of cases of preinvasive carcinoma.

Precancerous metaplasia presages the development of malignant neoplasia, but does so with less certainty than preinvasive carcinoma.

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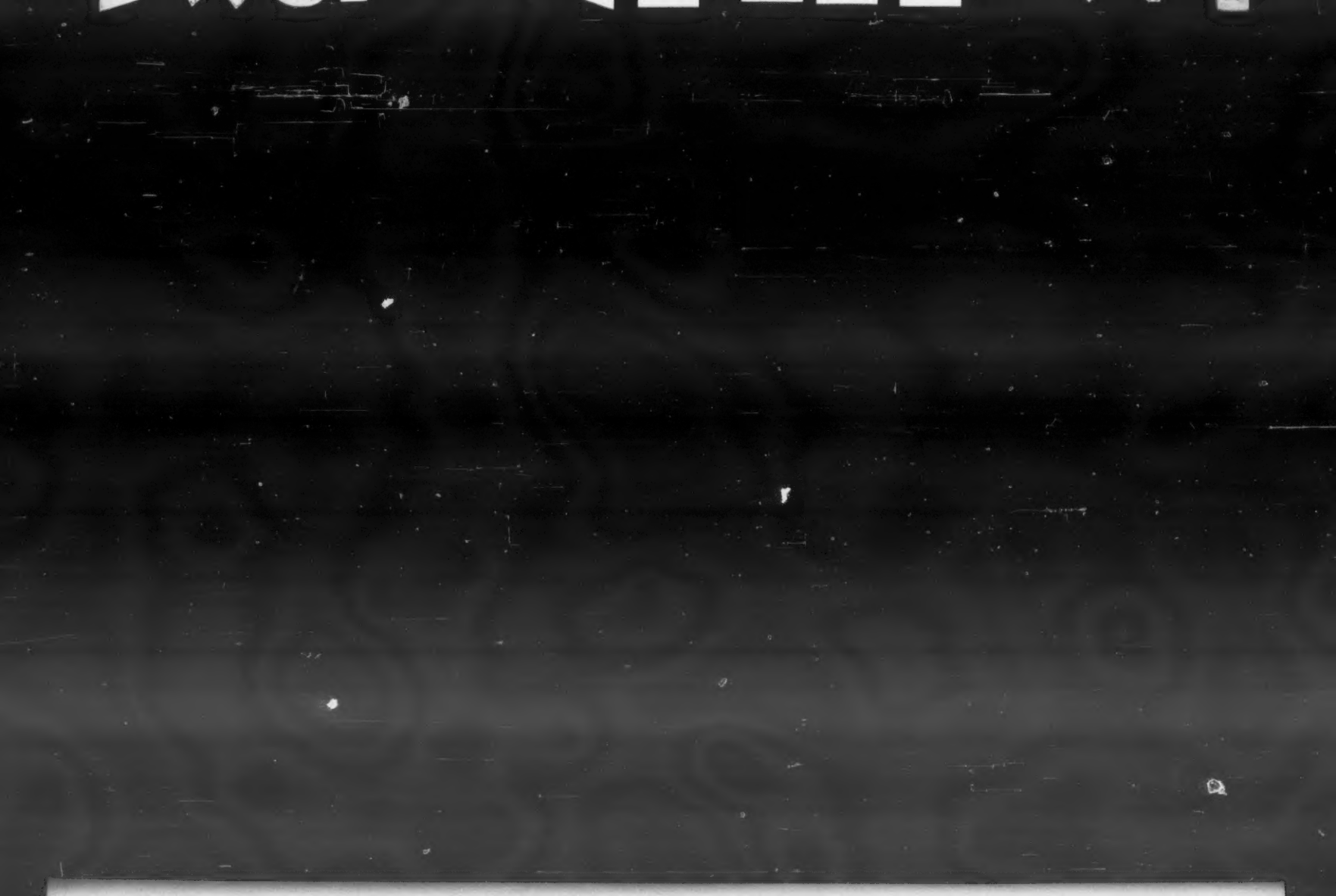
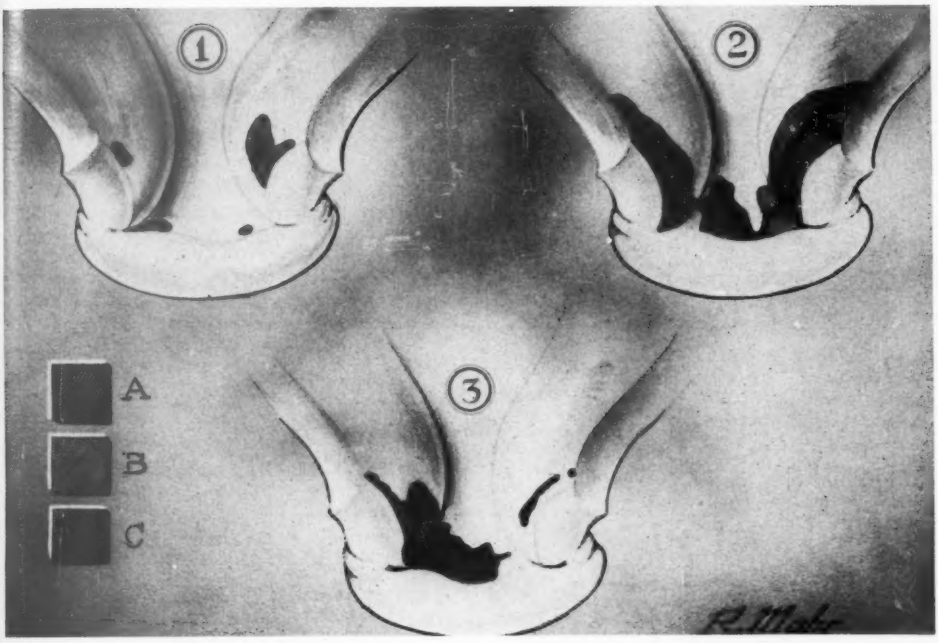
LEGENDS FOR FIGURES

FIG. 1. Artist's reconstruction of selected examples of the serial block survey carried out in group C. Diagram 1 represents multiple foci of preinvasive carcinoma and precancerous metaplasia. Diagram 2 indicates a broad, encircling plaque of preinvasive carcinoma, part of which is accompanied by a precancerous lesion. In diagram 3 both the multiple focus and broad plaque phenomena are shown and in addition there is an area of unsuspected invasive neoplasm. Of note are the limitation of these lesions to the endocervix and the freedom of extension to the vaginal surface of the cervix. Crosshatch (A) indicates preinvasive carcinoma; the fine stipple (B), precancerous metaplasia; and the coarse stipple (C), invasive carcinoma.

FIG. 2. An example of squamous metaplasia within the endocervical canal. There is an orderly pattern of cells and nuclei with well preserved polarity. × 200.

FIG. 3. Individual cell atypism. With a background of squamous metaplasia there are scattered cells exhibiting large, misshapen, hyperchromatic nuclei. × 160.





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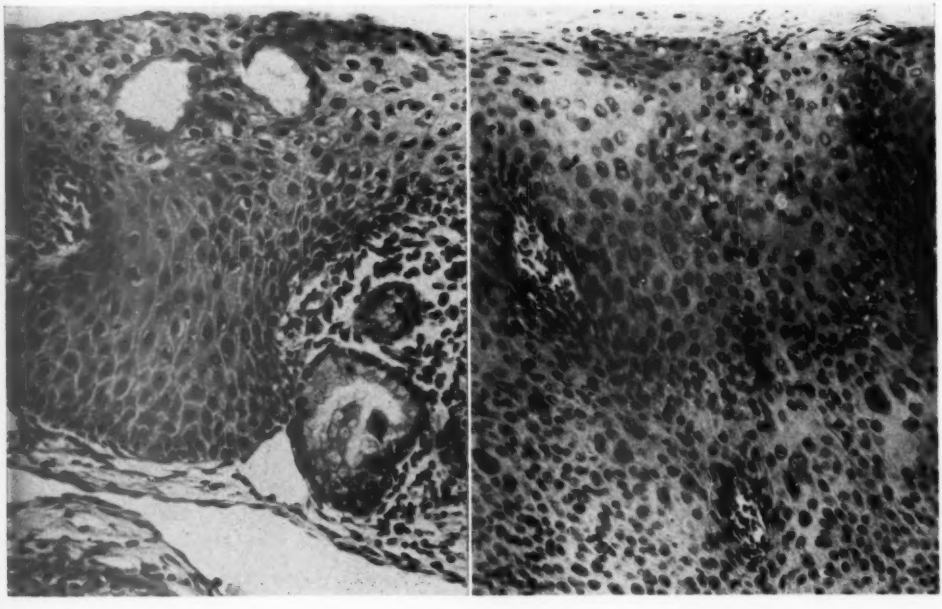

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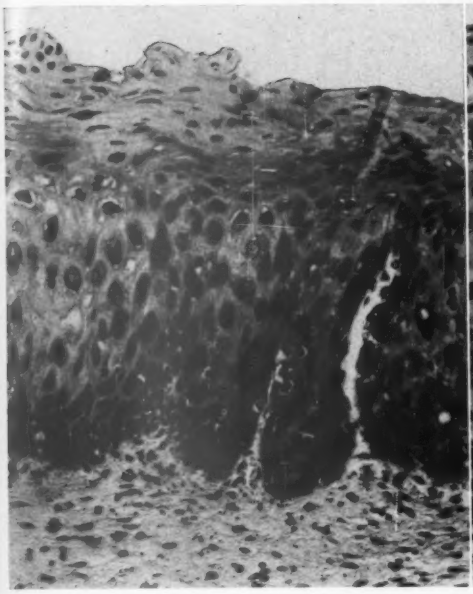
FIGS. 4 and 5. Precancerous metaplasia. There is marked loss of polarity among the cells intervening between the basal columnar and the surface layers. Nuclei are bizarre, hyperchromatic, have varied polarity, and occasionally exhibit mitotic figures. The cytoplasm remains abundant, eosinophilic, and the cells are polyhedral. The surface layer reveals restoration of polarity, the cells becoming oriented with the long axis parallel to the surface. Nuclei have lost atypism and appear effete. × 200.

FIGS. 6 and 7. Preinvasive carcinoma. Nuclear and cytoplasmic abnormalities continue throughout the entire depth of the mucosal epithelium. Cells are spindle-shaped and, although with varied nuclear polarity, are arranged with long axes perpendicular to the surface. Cytoplasm is scant, basophilic, and the nuclei are hyperchromatic with frequent mitotic figures. × 200.

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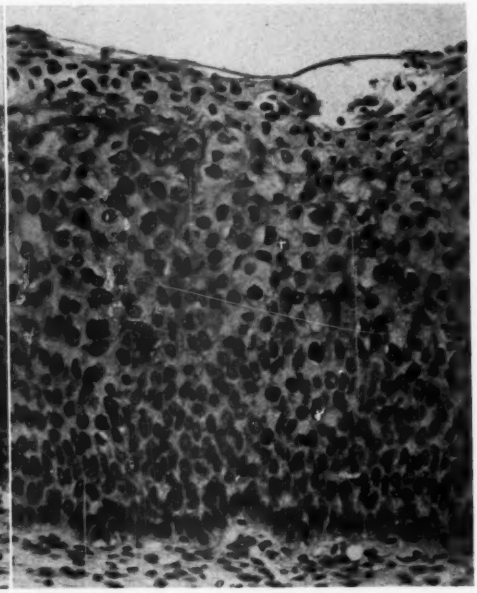

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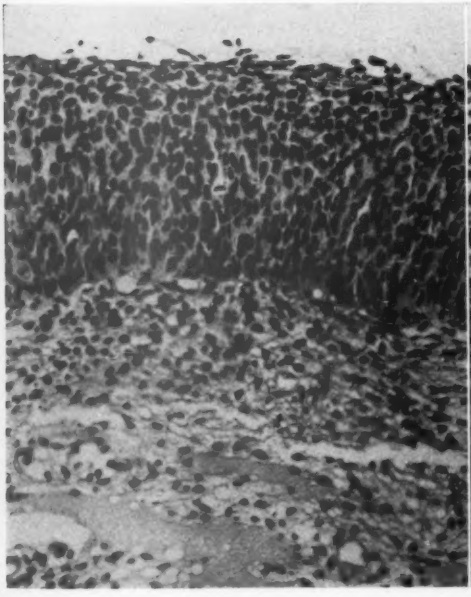

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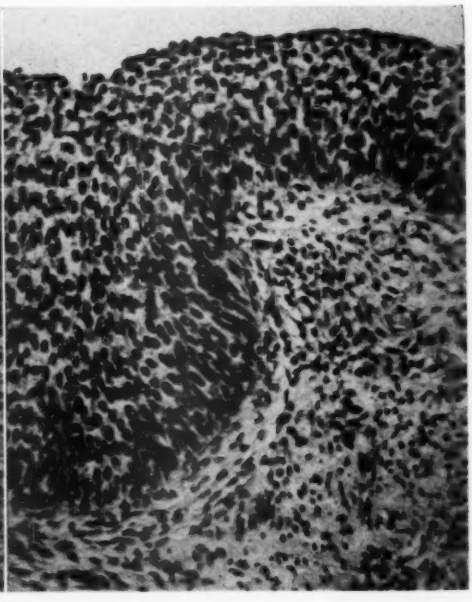

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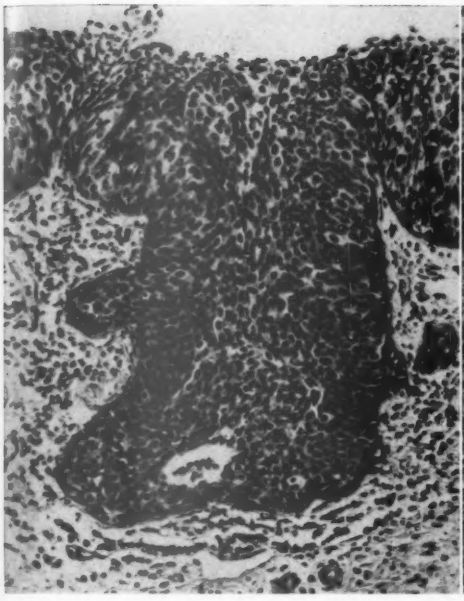
FIG. 8. Section from a hysterectomy specimen resected at 4½ months' gestation. There is preinvasive carcinoma with extension into a cervical gland. The basement membrane remains intact. × 160.

FIG. 9. A vaginal recurrence appearing 2 years after resection of the uterus from which Figure 8 was prepared. The photomicrograph demonstrates a submucosal nodule of frank squamous cell carcinoma. × 160.

FIG. 10. Cervical tissue secured for biopsy at 6 months' gestation. Preinvasive carcinoma and a decidual reaction in the subjacent stroma are shown. × 200.

FIG. 11. Invasive squamous cell carcinoma detected in the resected uterus from which the specimen shown in Figure 10 was obtained. Hysterectomy was performed at term because of uncontrollable post-partal hemorrhage. × 160.



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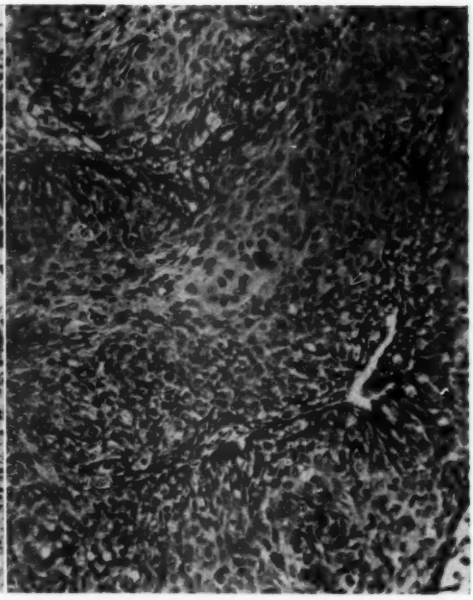

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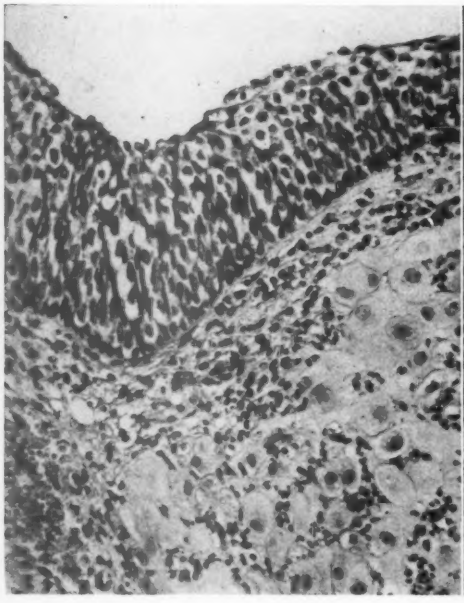

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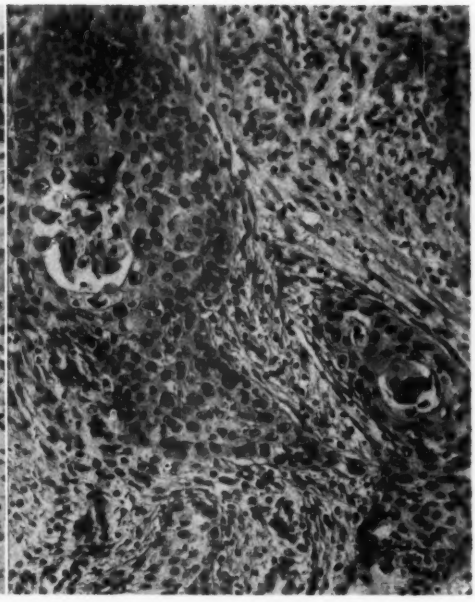

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THE RELATIONSHIP OF THE WEIGHT OF THE HEART AND THE CIRCUMFERENCE OF THE CORONARY ARTERIES TO MYOCARDIAL INFARCTION AND MYOCARDIAL FAILURE *

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One of the limiting factors of cardiac function is the blood supply to the myocardium, which in turn is limited by the capacity of the coronary arteries. We postulated that:

1. The sum of the internal circumferences of the main coronary arteries, as measured at necropsy, is indicative of the capacity of these vessels.

2. A relationship might become evident if the sum of the circumferences of the coronary arteries in correlation with the weight of the heart was studied in relation to the occurrence of myocardial infarction and cardiac failure.

3. The functional capacity of the heart could be roughly evaluated from the ratio of the heart weight to the sum of the circumferences of the coronary arteries.

The literature, reviewed from 1925 to the present, contains studies of the physiology of coronary circulation,[1,2] variations of the anatomical pattern of the coronary vessels,[3] cardiac hypertrophy, cardiac failure, and hypertension as related to coronary arteriosclerosis,[4-6] body weight-heart weight relations,[7] cachectic atrophy of the heart,[8] and myocardial infarction in the absence of coronary occlusion.[9-10] We have been unable to find reference to studies of the capacity of the coronary arteries and its relation to structural and functional abnormalities of the heart.

MATERIAL AND METHODS

The data were compiled from 130 consecutive necropsies, and from 29 additional necropsies selected on the basis of myocardial hypertrophy with or without myocardial infarction or cardiac failure. The right and left coronary arteries were opened lengthwise and their circumferences were measured in millimeters near their origins. The hearts, freed of blood and of parietal pericardium and with but a few centimeters of the great vessels attached, were weighed in grams.

For purposes of graphic presentation, an index was obtained by

* Supported by funds from the Nelson M. Percy Research Foundation, Chicago, Ill.
Received for publication, May 27, 1953.
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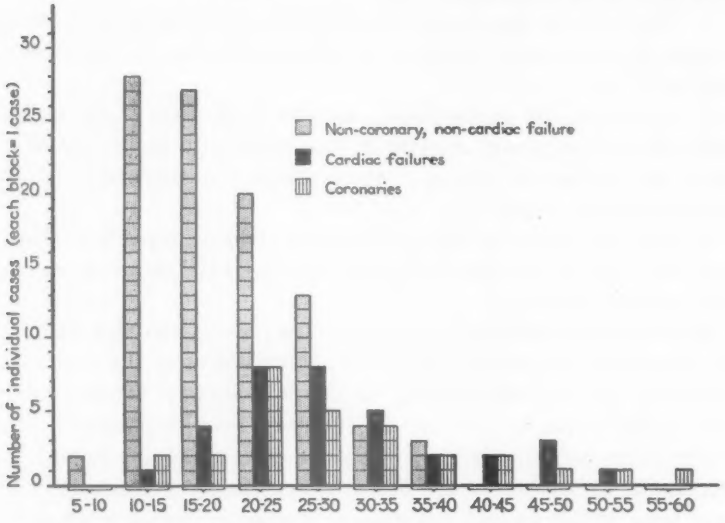
dividing the weight of the heart by the sum of the circumferences of the right and left coronary arteries. Thus, the larger the sum of the circumferences of the coronary arteries in proportion to the heart weight, the smaller the index. The cases were arranged in ascending order of the index and were placed in one of three categories; namely, those with recent myocardial infarction, those with clinical and anatomical evidence of myocardial failure, and those without clinical or



Text-fig. 1. Patients dying of non-cardiac diseases, cardiac failure, and coronary occlusion with myocardial infarction, distributed according to the index obtained by dividing heart weight in grams by the sum of the circumferences of the coronary arteries in millimeters. Each square represents one patient.

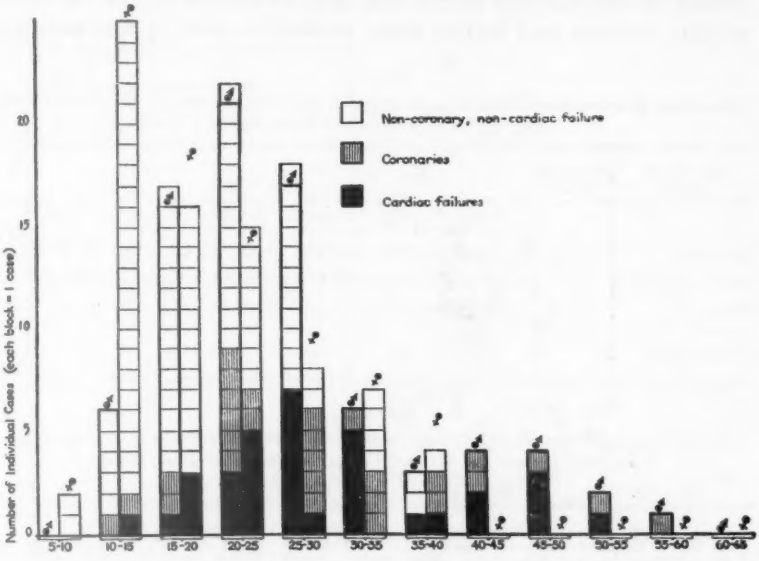
anatomical evidence of myocardial infarction or cardiac failure. The three categories were charted according to their indices and category (Text-fig. 1), according to their sex, index, and category (Text-fig. 2), and according to their age, index, and category (Text-fig. 3).

Results

There were 77 females (48.4 per cent) and 82 males (51.6 per cent) in the series. Of the 28 deaths as a direct result of coronary occlusion and myocardial infarction, 46.4 per cent occurred in females and 53.5 per cent occurred in males. Of the 34 deaths from myocardial failure, 32.3 per cent occurred in females and 67.6 per cent occurred in males. The non-cardiac group was made up of 53 females (54.6 per cent) and 44 males (45.3 per cent).

The effect of cachexia on the size of the heart has long been recog-

nized. Hellerstein and Santiago-Stevenson[8] found that the heart mass is reduced to a greater degree than the body mass in cachexia. We have observed that, with moderate weight loss, the weight of the heart does not appear to be materially affected. On the other hand, the atrophic heart of the cachectic individual is notably devoid of ather-



Text-fig. 2. The patients in the three categories as to causes of death have been separated as to sex and redistributed according to index range.

omatous plaques, while calcified plaques are present to the expected degree.

This series was studied from the standpoint of the nutritional status of each case as evaluated clinically and at necropsy. The cases were classified as emaciated (23.3 per cent), well nourished (54.0 per cent), and obese (22.7 per cent). The nutritional state appears to have a bearing on the index (Table I) in that more than 50 per cent of the patients having an index of 15 or less, and less than 10 per cent of those with an index greater than 30 were emaciated. Conversely, less than 10 per cent of the group with indices of 15 or less and 25 per cent of those with indices over 30 were obese. The emaciated made up a notably small percentage (7.1 per cent) of the cases of myocardial infarction, while the obese contributed disproportionately to the myocardial failure group (Table II).

The emaciated made up 61.5 per cent of the cases (8 of 13 cases) with hearts weighing 250 gm. or less and only 14.4 per cent of the

cases (16 of 111 cases) with hearts weighing more than 350 gm. (Table III). Obesity, on the other hand, was noted in only one patient whose heart weighed 300 gm. or less.

The non-cardiac group had indices ranging from 5 to 40 with the majority (58.7 per cent) of cases falling below 20, *i.e.*, the circumference of the coronary artery was large in comparison to the heart weight; 34.0 per cent had an index between 20 and 30, and only 7.2

TABLE 1

Percentage Distribution of Patients as to Apparent Nutritional State, for the Entire Group and for Those in Particular Index Ranges

Nutritional status	Percentage of series	Index range					
		0–15	16–20	21–25	26–30	31–40	41–60
Emaciated	23.3	54.5%(18)	21.2%(7)	13.9%(5)	19.2%(5)	5.0%(1)	9.1%(1)
Well nourished	54.0	36.4%(12)	42.4%(14)	63.9%(23)	61.5%(16)	55.0%(11)	90.9%(10)
Obese	22.7	9.1%(3)	36.4%(12)	22.2%(8)	19.2%(5)	40.0%(8)	0
Total cases		33	33	36	26	20	11

TABLE II

Comparison by Number and by Percentage of Patients with Myocardial Infarction, with Myocardial Failure, and Without Cardiac Disease, after Distribution as to Apparent Nutritional State

	Percentage of series	Myocardial infarction	Myocardial failure	Non-cardiac
Emaciated	23.3%	7.1%(2)	17.6%(6)	29.9%(29)
Well nourished	54.0%	78.6%(22)	47.1%(16)	48.5%(47)
Obese	22.7%	14.3%(4)	35.3%(12)	21.6%(21)
Total cases	159	28	34	97

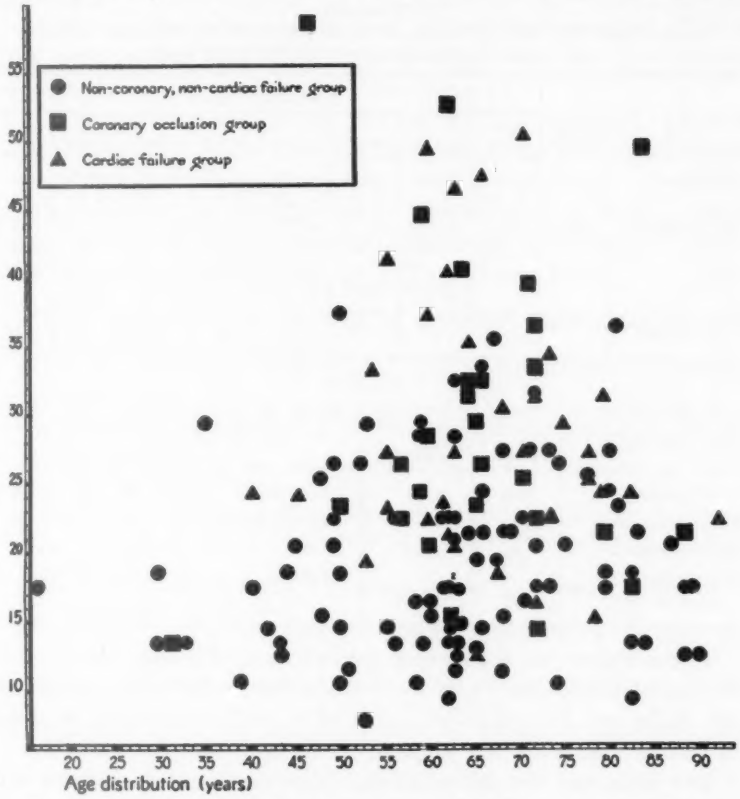
per cent were within the 30 to 40 range, with no cases having an index above 40. The indices of the cardiac failure group varied between 10 and 55; 14.7 per cent had an index below 20, 47.0 per cent were within the 20 to 30 range, 20.5 per cent in the 30 to 40 range, and 17.6 per cent were above 40. The acute coronary occlusion group had indices from 10 to 60 with 14.3 per cent below 20, 46.4 per cent were in the 20 to 30 range, 21.4 per cent were in the 30 to 40 range, and 17.6 per cent were above the 40.

The median index* for the entire series and for each category is given in Table IV.

DISCUSSION

The sum of the circumferences of the left and right coronary arteries

and its relation to the weight of the heart is a simple, albeit crude, objective method available at necropsy to indicate the potential blood supply to the myocardium. For the blood supply per unit of myocardium to remain constant, the capacity of the coronary arteries



Text-fig. 3. Scattergraph of all cases based on age and index range, with the three categories as to causes of death indicated by symbols.

must increase in proportion to any increase in myocardial mass. If this relationship is not maintained, the functional reserve of the heart must of necessity be reduced. Whether this occurs as the result of failure of the coronary arteries to keep pace with the normally growing myocardium, or as a result of failure of the coronary arteries to keep pace with the myocardium that is hypertrophied as the result of pathologic work demands, or narrowing of the coronary arteries due

* Statistical analysis was done by Mr. Philipp W. Zinkgraf, chief of research statistics.

to arteriosclerosis, the results are the same; namely, an increasing incidence of coronary occlusive disease with resultant myocardial infarction or myocardial failure. The validity of this principle has been demonstrated in the present study.

TABLE III

Comparative Distribution of Patients by Number and by Percentage According to the Weight of the Heart and Apparent Nutritional State

	Percentage of series	Heart weight in grams							
		250 or less	251–300	301–350	351–400	401–450	451–500	501–600	Over 600
Emaciated	23.3%	61.5% (8)	38.9% (7)	29.4% (5)	14.3% (4)	12.5% (2)	21.9% (7)	14.3% (3)	0
Well nourished	54.0%	38.5% (5)	55.6% (10)	35.3% (6)	57.1% (16)	56.3% (9)	50.0% (16)	66.7% (14)	71.4% (10)
Obese	22.7%	0.0	5.5% (1)	35.3% (6)	28.6% (8)	31.2% (5)	28.1% (9)	19.0% (4)	28.6% (4)
Total cases		13	18	17	28	16	32	21	14

TABLE IV

Median Indices as Computed for the Entire Series and for Each Component of the Series

	No. cases	Median index		No. cases	Median index
Entire series	159	22.2	Coronary	28	26.85
Consecutive cases	130	20.0	Cardiac failure	34	27.85
Selected cases	29	30.0	Male	82	23.65
Non-cardiac, non-coronary	97	18.45	Female	77	18.0

It was found that the smaller the index, *i.e.*, the larger the circumference of the coronary arteries in proportion to the heart weight, the less likely was the individual to die as a result of coronary occlusion or cardiac failure and the greater was his probable age at death.

The collateral circulation arising from the coronary arteries may also be influenced by the capacity of coronary arteries, *i.e.*, the larger the capacity of the coronary artery the more readily might a collateral circulation develop. Thus, even in the face of segmental narrowing or occlusion, the ultimate damage may be reduced by this factor.

SUMMARY

An index derived from the heart weight in grams divided by the sum of the circumferences of the right and left coronary arteries was applied to a study of cases of coronary occlusion with myocardial infarction, myocardial failure, and to non-cardiac patients who were necropsied.

The older the age group, the smaller the index tended to be.

Females tended to have smaller indices than males.

Of the non-cardiac group, 78.6 per cent had an index of 25 or less, 21.2 per cent were in the 25 to 40 index range, and none had an index above 40.

Of the cardiac failure group, 34.3 per cent had an index of 25 or less, 46.8 per cent were in the 25 to 40 index range, and 18.7 per cent were in the 40 to 55 range, with none above 55.

Of the acute coronary group, 42.8 per cent had indices of 25 or less, 39.3 per cent fell within the 25 to 40 index group, and 17.8 per cent were in the 40 to 60 index group.

The heart weight/coronary artery circumference is a rough index, obtainable at necropsy, of the functional capacity of the myocardium.

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THE CHANGING MORPHOLOGIC PICTURE OF ENDOCARDITIS SINCE THE ADVENT OF CHEMOTHERAPY AND ANTIBIOTIC AGENTS*

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Since the advent of chemotherapy and the use of antibiotics, the incidence and the appearance of vegetations on the cardiac valves, as seen at necropsy, have presented striking changes. A systematic study was undertaken of 5,033 necropsies performed from 1936 to 1946, and the results were compared with the findings on the heart valves in 3,643 post-mortem examinations from 1946 to 1951. The year 1946 was chosen as the date prior to which inadequate treatment or no treatment had been administered at this institution. This study was conducted from three different standpoints. The first approach included the over-all evaluation and the statistical summation of the bacterial and non-bacterial vegetations found on the heart valves during the periods before and during the present therapeutic era. The second approach dealt with the study of specific instances of bacterial endocarditis which showed an actual local effect of the therapeutic regime. The third aspect of the study, the experimental production of vegetations, was based on the generalities evolved from the first two studies and is being reported elsewhere (Marquiss and Angrist[1]).

ANALYSIS OF SERIES

Of 8,676 patients, 372 showed vegetations during the 15-year period. Only 5 cases of atypical verrucous endocarditis and 39 of acute verrucous rheumatic valvular lesions were found during the period. They were excluded from the statistical studies and are not included here. It is noteworthy that several acute rheumatic and Libman-Sacks' non-bacterial vegetations showed surface bacterial contamination and thus transition to vegetative bacterial endocarditis. Essentially, then, the comparative study presents classic bacterial vegetative endocarditis on the one hand and degenerative, bland, thrombotic, non-bacterial endocardiosis on the other. The clinical histories, gross protocols, and numerous gross specimens and photographs of the valvular lesions, as well as microscopic sections of nearly all of the vegetations were studied. Ordinary hematoxylin and eosin sections were used, with phospho-

* Presented at the Fiftieth Annual Meeting of the American Association of Pathologists and Bacteriologists, St. Louis, April 3, 1953.
Received for publication, June 19, 1953.

tungstic acid hematoxylin, elastica, and van Gieson's and MacCallum's stains as indicated. All of the valvular lesions had been seen by one observer, so that continuity prevailed as to the criteria of classification. The recovery of organisms in the bacterial forms and the actual administration of penicillin or other potent therapeutic remedies were established.

Several cases showed gross evidence of healing which could be attributed to the therapy instituted, although spontaneous healing might well have played a considerable part. Illustrative cases are presented in detail to show the range of variation in the actual bacterial lesions as effected by the therapeutic regimes. Completely healed and calcific valvular lesions could not always be correlated with a preceding episode of bacterial vegetative endocarditis because of the inadequate histories in many instances. Several cases presented very definite clinical evidence of past sepsis, with physical signs of valvular involvement at that time, and showed healed distorted valves at necropsy, indicative of the antecedent ulcerative and vegetative valvular lesion.

Findings and Illustrative Cases

Table I gives the over-all incidence of acute and subacute bacterial endocarditis by years and by patient age in decades. A total of 93 cases in 5,033 necropsies prior to 1946 is comparable to 17 instances in 3,643 necropsies in the 5-year period of the recent therapeutic era. There has been a drop in incidence from 1.85 per cent to 0.47 per cent, representing a fourfold decrease in the number of cases of bacterial endocarditis for these periods. The decreased incidence is most obvious in the younger age group. Table II gives the incidence of vegetations of degenerative non-bacterial endocardiosis (thrombotic non-bacterial endocardiosis) by year and age decade. It will be noted that the corrected incidence before 1946 is 1.97 per cent as compared with the corrected incidence of 4.47 per cent for the 5 years since the advent of the newer remedies. This is a two and one-half fold increase. This is shown graphically by Text-figure 1.

In Table III the incidence is indicated separately for the younger and older age groups. The decreased incidence in bacterial endocarditis and the increased incidence of thrombotic non-bacterial endocardiosis are seen in all groups, but most strikingly in the patients over 50 years of age.

In Table IV the occurrence of vegetations of thrombotic non-bacterial endocardiosis in sclerotic, rheumatic, and normal valves is shown. It will be noted that such occurrence in the younger patients

TABLE I

Incidence of Acute and Subacute Bacterial Endocarditis by Age and Years

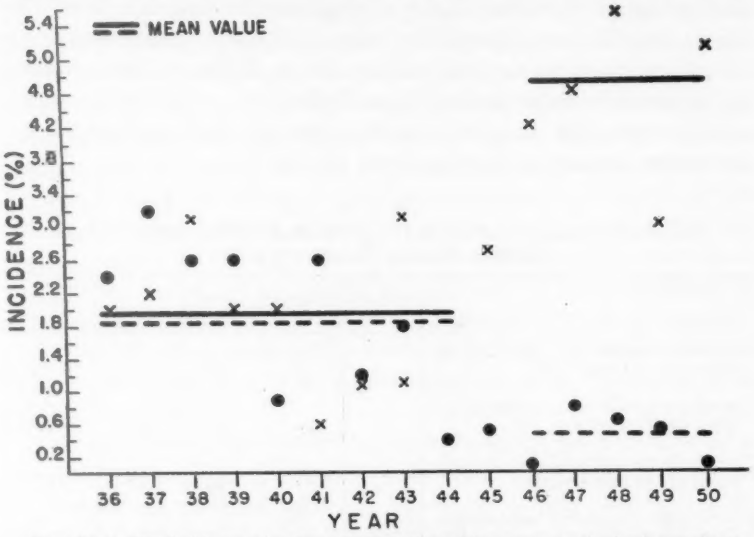
Age of patient	1936	1937	1938	1939	1940	1941	1942	1943	1944	1945	Total before 1946	incidence 1946	1946	1947	1948	1949	1950	Total after 1945	incidence after 1945
0–9	1			2							3	0.8							
10–19	1	3	4			3	1	1		1	14	8.4			1			1	2.2
20–29	2	1	1	8	1	2	1		1		17	5.7			1	1		2	1.8
30–39	4	1	5	1	2	3	3	1			20	5.0		2				2	1.1
40–49	2	3	1	1	2	2	1	5			17	2.5	1	2		2		5	1.3
50–59		4		2		2		1		1	10	1.1		1				1	0.1
60–69		2	1					1	1	1	6	0.6		1	3	1		5	0.6
70–79		1	1			1					3	0.4					1	1	0.1
80–89	1		1								2	1.1							
90+											0	0.0							
Unknown		1									1	17.0							
Total number	11	16	14	14	5	13	6	9	2	3	93		1	6	5	4	1	17	
Total number of necropsies	453	495	520	509	513	491	475	507	543	527	5033		621	724	813	776	709	3643	
Incidence	2.4	3.2	2.7	2.8	1.0	2.6	1.3	1.8	0.4	0.6		1.85	0.2	0.8	0.6	0.5	0.1		0.47

TABLE II

Incidence of Thrombotic Non-Bacterial Endocardiosis by Age and Years

Age of patient	1936	1937	1938	1939	1940	1941	1942	1943	1944	1945	Total before 1946	incidence 1946	1946	1947	1948	1949	1950	Total after 1945	incidence 1945
0–9		2	1	2					1		6	0.8				2		2	0.5
10–19	1	1	3		1	1		2	1		10	6.0			1			1	2.2
20–29		1	1	1		1	1		1		6	2.0	2	1	1	1	1	6	5.5
30–39	1		3	1	2		1	3		4	15	3.5	1	2	5			8	4.3
40–49		1	2	1	3		1	4	1	2	15	2.3	3	7	6	2	7	25	6.6
50–59	5	1	1	3	3	1		3	1		18	1.9	6	6	10	3	4	29	4.5
60–69	1	2	4	1	1		1	2	1	4	17	1.7	6	11	11	4	11	43	5.2
70–79	1		1	1			1			3	7	1.0	6	3	8	8	9	34	4.6
80–89		1						1		1	3	1.0	2	3	3	3	4	15	4.9
90+								1			1	50.0						0	0.0
Unknown		1									1	17.0						0	0.0
Total number	9	10	16	10	10	3	5	16	6	14	99		26	33	45	23	36	163	
Total number of necropsies	453	495	520	509	513	491	475	507	543	527	5033		621	724	813	776	709	3643	
Incidence	2.0	2.0	3.1	2.0	2.0	0.6	1.1	3.2	1.1	2.7		1.97	4.2	4.6	5.5	3.0	5.1		4.47

of all groups is not significantly different in the pre-penicillin and penicillin eras, whereas in the older age group considerable increase of thrombotic non-bacterial endocardiosis in all three sub-groups has oc-



Text-fig. 1. The incidence of endocardiosis, indicated by crosses, and of subacute bacterial endocarditis, indicated by solid circles. The solid line shows the mean incidence of thrombotic non-bacterial endocardiosis and the broken line that of bacterial endocarditis.

curred since the use of penicillin. This seems to implicate the aging process in all valves, including the rheumatic group, in the development of bland thrombotic non-bacterial endocardiosis.

TABLE III

Incidence of Thrombotic Non-Bacterial Endocardiosis and of Bacterial Endocarditis in Young and Old Age Groups Before and During the Penicillin Era

	Before 1946		After 1945	
	Under age 50	Over age 50	Under age 50	Over age 50
	%	%	%	%
Incidence of thrombotic non-bacterial endocardiosis	2.3	1.6	3.6	4.8
Incidence of acute and subacute endocarditis	3.2	0.8	0.9	0.2

REPORT OF CASES

Individual cases have been selected to illustrate the following variations: (1) bacterial vegetations in the process of healing, (2) completely healed bacterial endocarditis with resultant valvular de-

formity, (3) co-existence of thrombotic and acute bacterial endocarditis, (4) healed bacterial endocarditis with recurrence, (5) healed subacute bacterial endocarditis with resultant valvular deformity indistinguishable from those changes subsequent to rheumatic heart disease and non-bacterial thrombotic lesions, (6) the mechanisms of death in healed and healing bacterial endocarditis, including congestive failure due to severe valvular destruction and distortion, embolization, recurrence (re-infection of new thrombotic non-bacterial vegetation), exacerbation (inadequate therapy), and uremia.

TABLE IV

Occurrence, by Number of Cases, of Thrombotic Non-Bacterial Endocardiosis on Sclerotic, Rheumatic, and Normal Valves

	Sclerotic valves	Rheumatic valves	Normal valves	Total	Total necropsies
Thrombotic non-bacterial endocardiosis below age 50 before 1946	2	38	9	52	2239
Thrombotic non-bacterial endocardiosis below age 50 after 1945	3	24	12	42	1098
Thrombotic non-bacterial endocardiosis above age 50 before 1946	3	27	14	47	2796
Thrombotic non-bacterial endocardiosis above age 50 after 1945	37	57	26	121	2545

Case 1

C. R. was a Negro male, 46 years old, with no history of rheumatic fever, who was treated for subacute bacterial endocarditis at another hospital 4½ months prior to death. Positive cultures for *Streptococcus viridans* were obtained prior to 2 weeks of penicillin therapy (20,000 units every 3 hours). Following this his blood culture was sterile and he did well for 4 months. He was admitted to Queens General Hospital with severe headache, vomiting, and nuchal rigidity. His course was that of sepsis and he received penicillin (50,000 units every 2 hours) for 14 days.

The necropsy revealed evidence of healing bacterial vegetations on the aortic valve with rounded perforations of all cusps, indicative of old destruction undoubtedly related to the previous (healed) episode of bacterial endocarditis (Figs. 1 and 2). The mitral valve showed thrombotic non-bacterial endocardiosis with no evidence of bacterial contamination. Thickening of the edge was the only rheumatic stigma noted. No post-mortem cultures were taken. The immediate cause of death was rupture of a mycotic aneurysm of the left posterior cerebral artery. Microscopic examination of this lesion showed an organizing thrombus with old calcific masses, bacteria, and extensive suppurative reaction in the entire wall of the regional vessel. This was probably

embolic from the terminal valvular episode and the changes indicated more recent healing.

The aortic valve showed complex and variable histologic changes. Figure 3 is a camera lucida diagram of the distribution of valvular substance, fibrinoid, and oriented histiocytes. The collagen of the original valve showed degenerative changes (A) and a superimposed fibrosing organizing vegetation toward the base of the cusp (B). Toward the perforation, the edge of which is shown at D, the valvular collagen showed extensive and marked degeneration and necrosis. Figures 4 and 5 are low and high-power photomicrographs from the perforation site and show superficial cellular exudate over fibrin and fibrinoid material. The valve shows extensive proliferation of Anitschkow-like myocytes and histiocytes with distinct evidence of palisading in zones apposed to fibrinoid material. Figure 6, through an adjacent section of the site of perforation, shows prominent histiocytic reaction, degenerative collagen, and an Aschoff-like nodule in relation to some fibrinoid material.

This heart presents a healed bacterial endocarditis occurring on a relatively normal valve with old perforations remaining as evidence of previous sites of destruction of the valve. It also illustrates a recurrence of subacute bacterial endocarditis with some effect of recent therapy.

Case 2

I. P. was a white female, 33 years old, who was admitted with a history of rheumatic fever at age 7 and an episode of sepsis 1 year previously. The results of blood cultures at that time are not known, but 15,000,000 units of penicillin were administered. During the year prior to admission she developed symptoms of congestive failure. There was recurrence of sepsis with petechiae, and the patient was admitted to Queens General Hospital where she received penicillin (800,000 units daily) for 6 days. Blood cultures, ante mortem and post mortem, were negative.

The necropsy revealed chronic rheumatic valvulitis of the mitral valve with superimposed acute bacterial vegetations on the anterior cusp. In addition, there was an old rupture of a chorda tendinea of the posterior cusp in a region of old scarring (Fig. 7). A typical bacterial vegetation is shown at arrow 2, and the coiled ruptured lower portion of the chorda at arrow 1. A free dangling chorda is also seen merging with the thickened edge of the valve. No evidence of acute inflammation was seen at any of the sites of rupture of the chorda. This case presents a previously healed subacute bacterial endocarditis with resulting ruptured chorda, the patient developing a recurrent attack of subacute bacterial endocarditis at another site on the mitral valve.

Case 3

C. G., a white male, 16 years of age, gave a history of acute rheumatic fever at age 5. One year prior to death he developed subacute bacterial endocarditis (non-hemolytic streptococcus) and received penicillin (300,000 units every 3 hours for 3 months) and aureomycin. Subsequently he developed severe congestive failure for which he was admitted to Queens General Hospital.

The necropsy revealed severe rheumatic involvement of all valves. A calcified vegetation at the base of the non-coronary aortic cusp undoubtedly represented the completely healed original focus of subacute bacterial endocarditis (Fig. 8). Considerable difficulty would have been encountered in identifying the distorted valve as a result of previous subacute bacterial endocarditis without the detailed history.

Case 4

E. S. was a white male, 40 years old, with a history of chorea in childhood. He was admitted to Queens General Hospital 5 months prior to death with sepsis and cutaneous petechiae. Blood cultures were repeatedly sterile or positive for *Staphylococcus aureus,* coagulase negative. He received penicillin for 6 weeks (2.4 million units) and was discharged apparently well, only to be readmitted 3 months later with severe abdominal pain and evidence of congestive failure (dyspnea, cyanosis, 3 plus peripheral edema). He expired 1 day later.

The necropsy revealed chronic rheumatic valvulitis with aortic and mitral stenosis. A large vegetation of the aortic valve on the left coronary cusp, from which *Str. viridans* was cultured, showed microscopic evidence of healing subacute bacterial endocarditis, with encapsulated calcification, fibrosing granulation tissue, and some endothelialized clefts. Thrombotic non-bacterial vegetations were present, some showing calcification (Fig. 9). The immediate cause of death was acute infarction of the large bowel from a bland embolus in the middle colic artery. Old infarcts were seen in the kidneys, spleen, and brain. This case shows evidence of healing with recurrence or exacerbation.

Case 5

E. S. was a white male, 30 years old, who was admitted 7 months prior to death when a diagnosis of subacute bacterial endocarditis was made. The physical findings and embolic phenomena made this diagnosis a compelling one, although blood cultures were persistently negative. He received penicillin for 2 months (300,000 units every 3 hours). He was readmitted 2 months later with progressive congestive failure and uremia.

The necropsy revealed distortion of the aortic valve, with almost complete destruction of one cusp, and a mulberry-like, superimposed, protuberant and calcified mass on the non-coronary cusp. There was

no evidence of acute inflammation. The kidneys showed intracapillary glomerulonephritis. This, then, presents a completely healed subacute bacterial endocarditis with loss of function of the aortic valve.

Case 6

W. H., a white male, 31 years of age, was known to have had rheumatic fever since the age of 14. He was admitted to another hospital 8 months prior to death where a diagnosis of subacute bacterial endocarditis was made. Blood cultures were positive. He received penicillin therapy (200,000 units daily for 8 weeks), and was discharged with negative blood cultures. He had a recurrence of subacute bacterial endocarditis 5 months prior to death. Blood cultures again were positive for *Str. viridans* and he again received intensive penicillin therapy (500,000 units daily) for 3 months. He gradually developed severe cardiac failure and expired.

The necropsy revealed chronic rheumatic distortion of aortic and mitral valves. There was complete destruction of the aortic cusps with superimposed non-bacterial lesions. This case represents an example of completely healed recurrent subacute bacterial endocarditis with no evidence of activity. The patient died in congestive failure because of valvular destruction.

Case 7

M. R., a white female, 65 years old, with no history or previous diagnosis of rheumatic heart disease, was hospitalized for 5 days during which time she gave evidence of sepsis and received penicillin (500,000 units every 3 hours) for 3 days.

The necropsy revealed chronic rheumatic deformity of all four valves. Thrombotic non-bacterial vegetations, with no evidence of bacterial contamination, were found on the aortic, pulmonic, and tricuspid valves. The mitral valve showed an acute bacterial vegetation. Bacteriologic studies were not done. This case is one in which multiple thrombotic non-bacterial vegetations developed on previously damaged valves with bacterial infection of one giving rise to bacterial endocarditis.

Case 8

G. B. was a white male, 22 years of age. He was known to have had rheumatic fever since age 7, and developed subacute bacterial endocarditis 6 months prior to death. Blood cultures were positive for *Str. viridans* and he received intensive penicillin therapy (2,800,000 units daily) for 3 weeks. Following this, blood cultures were negative, but progressive congestive failure developed. Terminally he developed fever for 2 days, the blood culture remaining sterile.

The necropsy revealed severe rheumatic involvement of the aortic and mitral valves with numerous protuberant calcified vegetations and superimposed non-bacterial thrombotic verrucae. Microscopically there was extensive dense fibrosis with calcification. No active inflam-

mation was seen. This case is an example of complete healing which at necropsy presented no pathognomonic evidence of previous subacute bacterial endocarditis.

Case 9

E. R., a white male, 26 years old, had a history of rheumatic fever since the age of 12. He had one episode of sepsis 5 months prior to death. Blood cultures were sterile but the clinical picture was very suggestive of subacute bacterial endocarditis. He received intensive penicillin therapy for 1 month. One month later he had a recurrence of subacute bacterial endocarditis, and at that time *Str. viridans* was cultured from the blood (sensitive to 0.062 units of penicillin). He received 1,000,000 units of penicillin daily for 28 days. On his final admission, there was again sepsis, with blood cultures positive for *Str. viridans*. Congestive failure was prominent and he expired after 1 week of penicillin therapy (300,000 units every 2 hours).

The necropsy revealed severe old rheumatic involvement of all valves. The aortic valve was the seat of a subacute bacterial vegetation, which showed evidence of acute inflammation superimposed on an indolent healing lesion. *Str. viridans* was cultured from the vegetation on the aortic valve. This is a case of healing subacute bacterial endocarditis, demonstrating an exacerbation with superimposed inflammatory reaction.

Discussion

The obvious clinical diminution of all forms of bacterial endocarditis finds expression in the incidence at necropsy. This can be ascribed to several mechanisms. Avowed and even advanced cases are treated effectively and cured.[2] Early cases, detected or suspected soon after onset, quite readily are rendered sterile and never become symptomatic, and the diagnosis never is definitely established. Little or no evidence of such early involvement persists at necropsy. More important in accounting for the lowered incidence is the undoubted beneficial effect on known foci productive of sepsis of present potent remedies, which are used as soon as the presence of such hazards is established. These include septic pyelonephritis, cellulitis, and thrombophlebitis, as well as chronic sinusitis and apical dental abscesses. All such potential or prevailing sources of bacteremia are now diagnosed earlier and treated more effectively. As a result, the former repeated or long-continued invasion of the blood stream no longer prevails. Similar benefit is derived from prophylactic administration of antibiotics whenever a tooth is to be extracted, a sinus invaded, or an infected site incised. The over-all bacteremic seeding of the circulation has thus been significantly curtailed and this must have its effect on the prevention of bacterial endocarditis, no matter what the exact mechanism of the origin of bacterial vegetations may be.

If bacterial localization within the valve were the initial step in the mechanism of the formation of vegetations, one would expect that the over-all total of *all* vegetations would diminish in equivalent and similar fashion. It is obvious from the data that there has been a simultaneous increase in bland thrombotic non-bacterial endocardiosis in all categories since the advent of penicillin, corresponding with the decrease in the bacterial forms of the disease. This suggests that the thrombotic lesions do not now become infected to give origin to the bacterial vegetations. The increase in the number of cases with thrombotic non-bacterial endocardiosis (over and above the number accounted for by the failure of many such non-bacterial vegetations to become transformed into bacterial lesions as heretofore) is to be accounted for on the basis of increasing average age of the population and the survival of those with old sclerotic, rheumatic, and other fibrotic endocardial sites, known to be favorable for such metabolic alteration of collagen and for the formation of verrucae of endocardiosis.

The evidence for completely healed subacute bacterial endocarditis is necessarily inadequate and indefinite at necropsy. As might be expected, such healed valvular lesions often have final changes attributable to pre-existing bacterial vegetations incorporated in the still earlier scars of the valve. This is particularly true for the distorted rheumatic leaflets. Similarly, in their final stages, vegetations of calcific endocardiosis will look exactly like calcified bacterial lesions.

The illustrative cases were selected for the rather wide range of changes encountered. Moore[3] has detailed the pathologic findings in penicillin-treated bacterial endocarditis, noting the several steps in the healing process. Identical evidences of such healing have been noted in our cases of bacterial endocarditis, both acute and subacute, in which the patients died during therapy because of incidental embolization, uremia, decompensation, or other cause. Rosenblatt and Loewe,[4] Kaplan *et al.*,[5] Saxén,[6] Correll *et al.*,[7] Geiger and Durlacher,[8] Rapoport and Ellis,[9] and others have studied the morphology of treated vegetations. The inflammatory reaction takes on a more indolent character in areas, and fibroblastic proliferation and fibrosis are more evident, with an appearance of delimitation and encapsulation of necrotic foci and calcific zones. Some such foci suggest old entrapped bacterial colonies. More Anitschkow cells, histiocytes with a distinct tendency to parallel arrangement and perpendicular orientation to the edge of valve and vegetation, and more widely distributed round cells, are present. The isolated layers of the vegetation noted by

Moore, including the surface fibrosis, were not seen in most of the valves studied. Rather, complex irregular placement of zones of necrosis, inflammation, healing granulation tissue, and fibrosis, and areas of freshly formed or organizing bland vegetations of thrombotic non-bacterial endocardiosis, suggested strongly a variable ebb and flow of the healing process.

An important component of the histologic picture were the lesions of organizing thrombotic non-bacterial endocardiosis in the original bacterial vegetation, and also newly formed thrombotic non-bacterial vegetations on valve areas contiguous to the original bacterial lesion. Often the "ooze" of altered collagen and fibrinoid material was found superimposed on a bacterial vegetation which was well on its way to healing. A similar appearance often was seen to involve distant endocardium, particularly at scarred sites, independent of bacterial vegetations.

The thrombotic non-bacterial portion may be found with and without organization. The intimation exists that the metabolic fault that initiates the vegetation is persisting in such cases. This may well explain the failure of such cases to respond, especially with organisms isolated which are of demonstrated sensitivity to the anti-bacterial agents used. It is as though the mechanism for the continued formation of the basic lesion available for contamination remained, and that it was reinfected by the same or other organisms. The hazard of recurrence of subacute bacterial endocarditis from the same organism or from another, has been noted frequently by clinicians.

Lillehei *et al.*[10] have noted marked hypertrophy of the adrenal glands in their dogs with experimental endocarditis produced by A-V shunts. Selye[11] has produced endocarditis in rats by stress, which affects the adrenal glands particularly. Highman and Altland[12,13] have produced endocarditis by means of stress induced by placing rats under conditions equivalent to high altitudes and have found hypertrophy of the adrenal glands. The effect of ACTH and cortisone on collagen disturbances has a meaning on this basis. Pitressin has been used to produce vegetations experimentally.[14]

Nutritional factors have long been associated with endocardiosis, as the synonyms "marantic" and "cachectic" indicate. Vitamin C has long been thought to have a part in collagen metabolism, and Rinehart and Mettier[15] have used deficiency states to produce lesions like those of rheumatic fever in guinea-pigs. The known increase of endocarditis as a result of protein starvation during the war,[16,17] and the

demonstrated changes in total protein and in its electrophoretic pattern[18,19] in endocarditis have been stressed. The initial lesion of endocarditis, its progression, and its extension may well represent an underlying metabolic disturbance of collagen in which endocrine and nutritional factors play a part. The vegetations of thrombotic nonbacterial endocardiosis are a frequent terminal finding at necropsy because the last lethal illness tends to initiate the depleted state, giving rise to the metabolic fault in the collagen that causes them. Thus, they may be expected to be demonstrated as fresh incidental lesions having no apparent clinical significance.

This fundamental metabolic change in endocardiosis contributes to the total bulk of the vegetation in no small measure. The impression is gained that calcification seen in the healed lesions is not to be ascribed to the calcification of bacterial colonies or of necrotic valvular tissue alone, but often to the calcification of such sites of endocardiosis which can be quite extensive. Some of the basophilic, non-calcified material in vegetations may well represent altered nucleoprotein or glycoprotein material or the detritus of exudate, both cellular and protein.

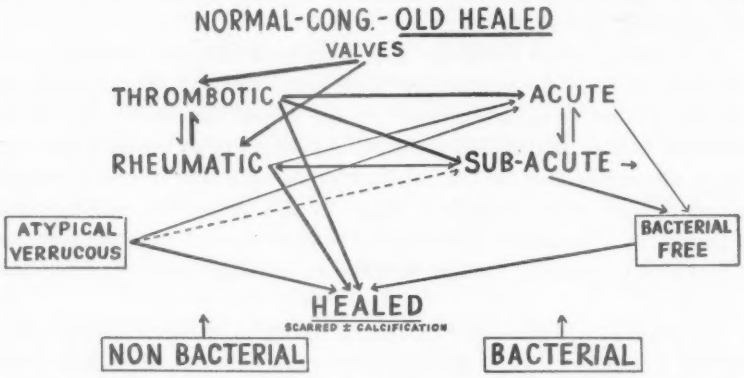
An important aspect of the treated bacterial valvular lesion is the finding of areas in the vegetation that show features of transition to vegetations of non-bacterial type and verrucae like those of active rheumatic fever. The cellular reaction often shows the palisading which is typical of the latter. The impression is gained that such an appearance in known rheumatic cases may be a non-specific connective tissue reaction or may represent an organizing or healing process in such lesions of endocardiosis, rather than indicating a recent rheumatic attack.[20]

Similar transitional appearances and manifestations of healing were seen in valvular lesions of subacute bacterial endocarditis long before the present therapeutic era. Libman[21] stressed this healing tendency. Von Glahn and Pappenheimer,[22] Clawson,[23] and others emphasized the features of identity in rheumatic fever and subacute bacterial endocarditis, and interpreted these as evidence for a distinct link between the two diseases. Similar appearances have been seen in treated and untreated valves without history or gross or histologic evidence of rheumatic fever. This so-called rheumatic histologic reaction probably is often no more than a mild indolent cellular response, such as is seen occasionally at the base of older vegetations of endocardiosis.

It is significant that such transitional morphologic appearances are

now more widespread and more common in bacterial endocarditis that has been under treatment for some time. The impression is obtained from the study of both the acute and subacute forms of bacterial endocarditis that they now approach one another in appearance. Text-figure 2 gives the actual transitions encountered in our experience.

Frank bacterial vegetative lesions of considerable bulk can go on to final arrest, calcification, and fibrosis, giving rise to equivalent distortion of the valve cusps and thus accounting for the changes seen at

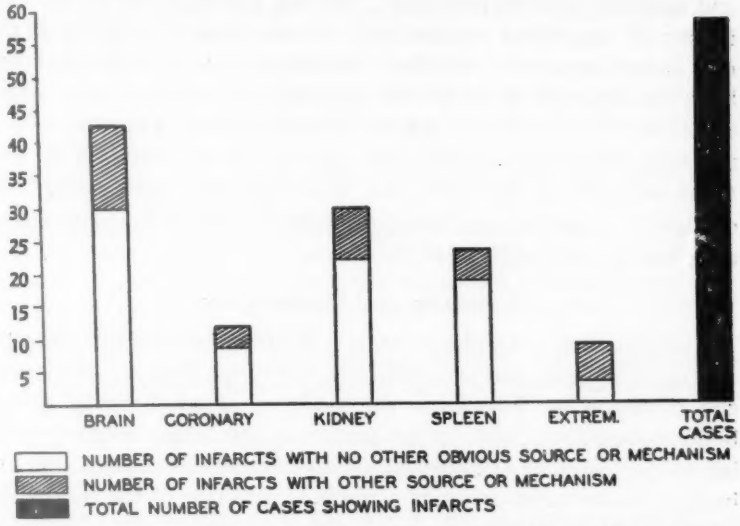


Text-fig. 2. Graphic representation of transitions seen in valvular vegetations. The intensity of the connecting arrows indicates the relative frequency of such transitions, as they may occur whether the valves were originally normal, congenitally deformed, or the site of old healed lesions.

necropsy long after the infectious episode. Many have suggested that calcific aortic stenosis may represent such healed bacterial vegetations. The similarity of their external forms on occasion prompts this consideration. Markedly distorted aortic and mitral valves are encountered too often at necropsy to be caused only by healed bacterial vegetations. Rather, it is the more frequent non-specific excrescences of endocardiosis which are quite similar in form, and just as liable to calcification and fibrosis, that account for the usual calcific aortic stenosis. The prevention of their bacterial contamination permits a summation of calcific and fibrotic changes over a longer period. It is our impression that the inherent metabolic change in the valvular collagen that occurs with age is more significant than any other mechanism in producing vegetations of thrombotic non-bacterial endocardiosis with resultant calcification.

For many years, embolization has been stressed in bacterial endo-

carditis. That bland emboli and non-suppurative infarcts appear in all forms of bacterial endocarditis, including the most septic virulent cases, has been well known. This was well established before the present therapeutic era, and was attributed to sterile non-bacterial portions of the vegetation, or to the heightened immunity at the local site of embolization. The morphologic study of valves with bacterial vegetations, particularly treated valves, suggests that the sources of such emboli for bland infarcts are the very common areas of recent accretion of bland vegetations of thrombotic non-bacterial endocardiosis.



Text-fig. 3. Number of infarcts in 272 cases of thrombotic non-bacterial endocardiosis.

Such vegetations are usually very superficial, with slight and very slow development of a basilar reaction of organization. Therefore, they are loosely adherent and readily dislodged. This is confirmed by the frequency with which pure thrombotic non-bacterial vegetations on the aortic and mitral valve are the source of embolic episodes. The difficulty in finding the site of origin, once such a vegetation is dislodged, and in identifying the embolus in the superimposed thrombus as such, hides the true mechanism; and the diagnosis of thrombosis is made too often. Both smaller and larger lesions of endocardiosis can and do produce emboli more frequently than has been indicated heretofore (Text-fig. 3).

Antibiotic and chemotherapy have been very effective in controlling all forms of bacterial endocarditis, and the resulting "brake-like" ef-

fect on the entire pathologic process helps to elucidate the healing changes and the mechanisms of formation and extension of vegetations. The processes of healing, calcification, and resolution of the inflammatory reaction,[21] similar to those seen before the modern therapeutic era, have been rendered more prominent and more amenable to study and understanding. The lesion of thrombotic non-bacterial endocardiosis is not to be considered an incidental terminal manifestation of academic interest only, but a highly significant one for the understanding of the morphology of all forms of endocarditis and their intermediate transitions. Healing always occurs in the vegetations of thrombotic non-bacterial endocardiosis, if uninfected, and now is seen commonly with bacterial vegetations. Uninfected thrombotic non-bacterial endocardiosis is completely asymptomatic unless embolism, calcification, or marked fibrosis occurs. Thrombotic non-bacterial endocardiosis is the key, central, primary lesion in all bacterial vegetations, important for their initiation, progression, and contiguous extension, and also contributes to their final appearance after healing and calcification.

Summary and Conclusions

There has been a definite *decrease* in bacterial endocarditis since the advent of antibotic and chemotherapy. There has been a simultaneous and corresponding *increase* in the incidence of non-bacterial thrombotic endocardiosis. This implies control of the factors formerly leading to the infection of such lesions, and favors the concept that the bacterial lesion often arises as a result of infection of the thrombotic non-bacterial vegetation.

The known tendency toward healing of subacute bacterial endocarditis is enhanced markedly by modern therapy. Transitional lesions between the basic forms of endocarditis are seen with increasing frequency, and the basic individual forms approach each other more closely. Distorted valves with perforations and bulky calcific vegetations occasionally represent healed bacterial vegetations, but more often are the result of healed degenerative endocardiosis.

Non-bacterial vegetations constitute an important source of embolization, often leaving little evidence at the site of origin on the valve. Some of the bland infarcts encountered in septic endocarditis originate from such non-bacterial sites.

The valves previously damaged by rheumatic distortion, aging, or any mechanism favoring fibrotic scarring and hyalinization, show a greater tendency to collagen degeneration, thereby accounting for the

known frequency of vegetations of all forms on such damaged valves. Thrombotic non-bacterial endocardiosis is linked with a fundamental metabolic fault of collagen metabolism in which the endocrine system, the aging process, and the nutritional status seem to play a part. This implies that the original morbid anatomical distortion in *all* endocarditis is a biochemical one still to be elucidated.

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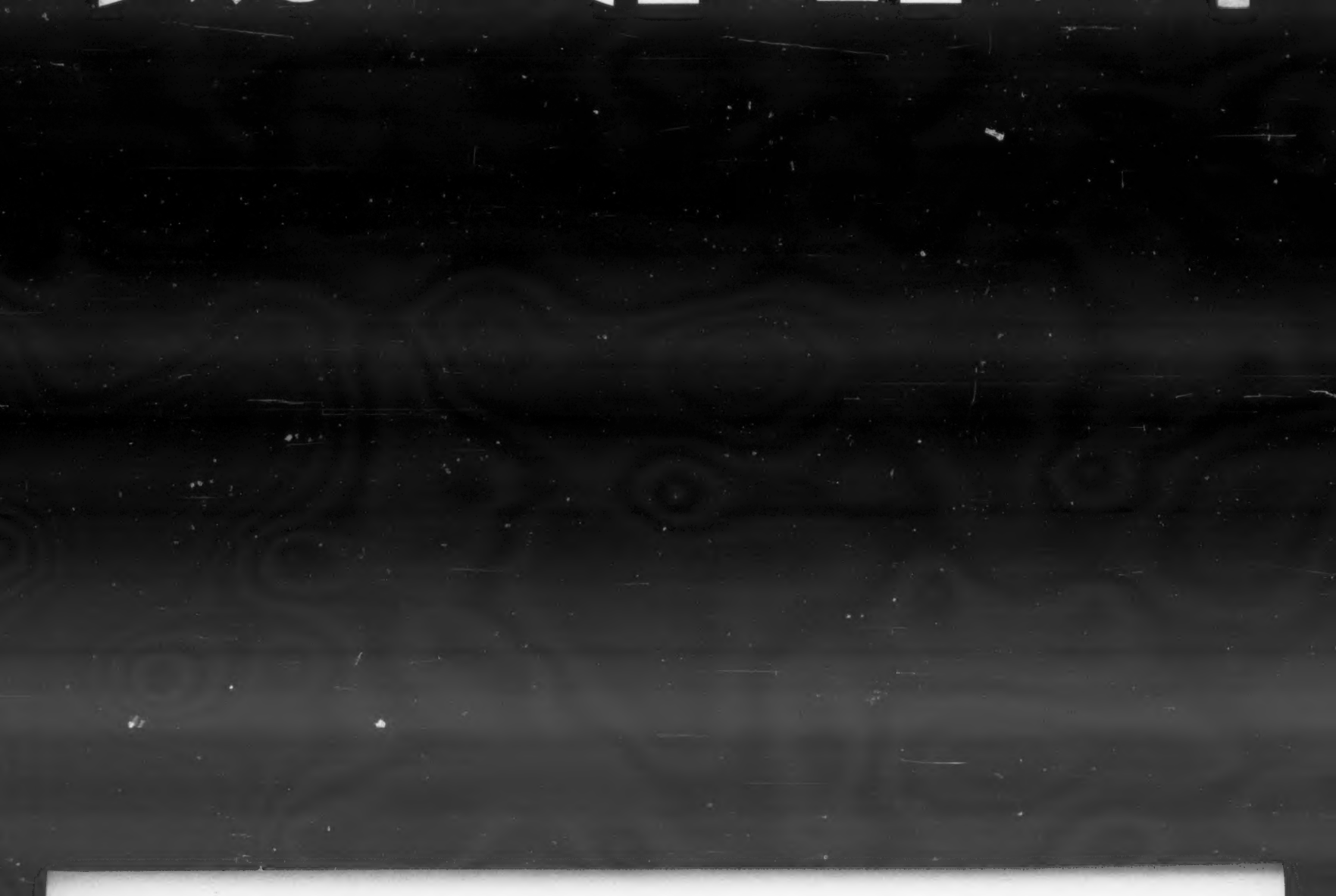
LEGENDS FOR FIGURES

FIG. 1. Case 1. Aortic valve showing rounded, scarred perforations with thickening of the cusps, and rounded, elevated nubbins along the edges of perforations, shown well on the non-coronary cusp.

FIG. 2. Case 1. Matchsticks have been inserted in the perforations. There is a whitish scarred margin about the perforation of the right coronary cusp and fresher vegetations about the opening in the non-coronary cusp.



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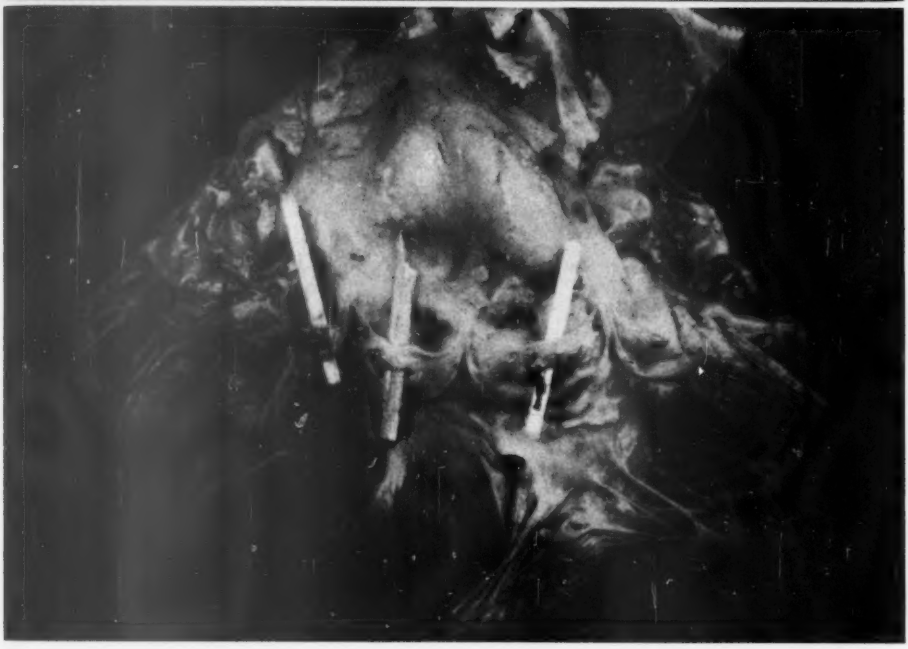

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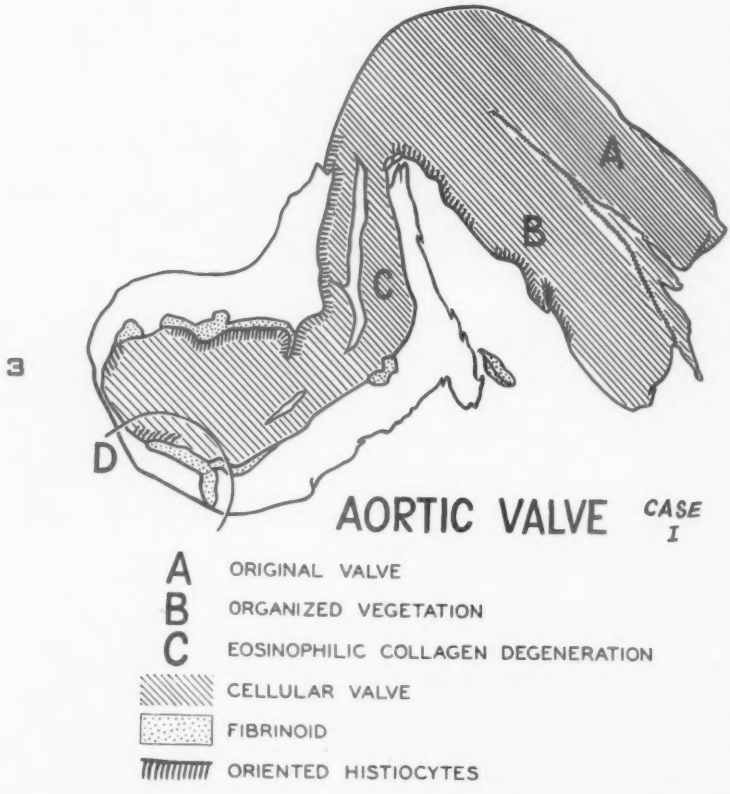
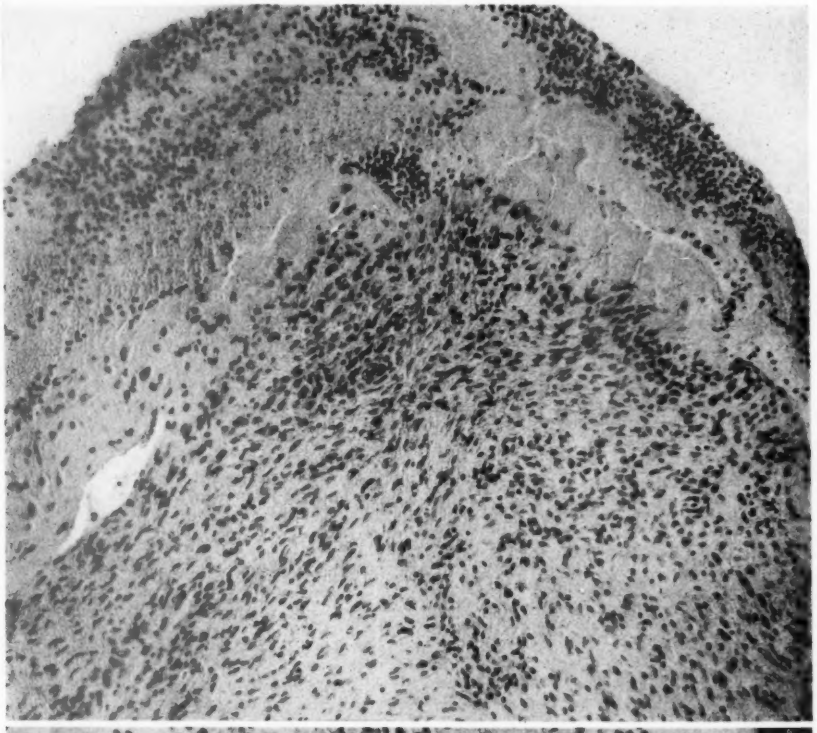
FIG. 3. Case 1. Camera lucida diagram of a microscopic section of an aortic valve cusp, showing oriented arrangement of the original valve, organized vegetation, fibrinoid, and fibrin. D indicates the region of perforation as illustrated in Figure 4.

FIG. 4. Case 1. Line of perforation (D in Fig. 3) showing superficial acute inflammatory exudate with fibrinoid material and fibrin overlying the cellular valve. Hematoxylin and eosin stain. × 40.

FIG. 5. Case 1. The same area (D) as seen in Figure 4, showing the palisading of histiocytes in zones apposed to fibrinoid material. Hematoxylin and eosin stain. × 180.





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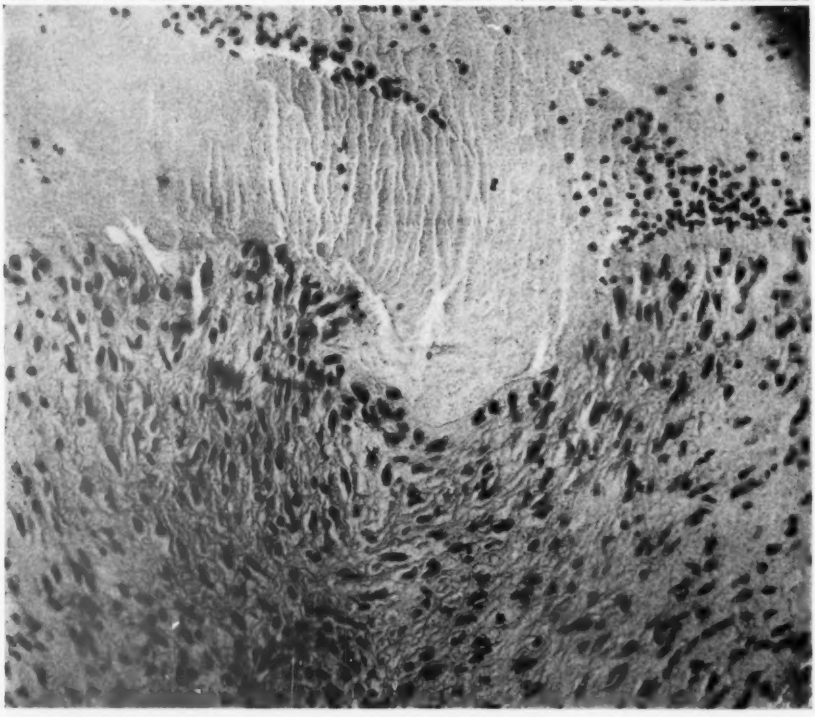

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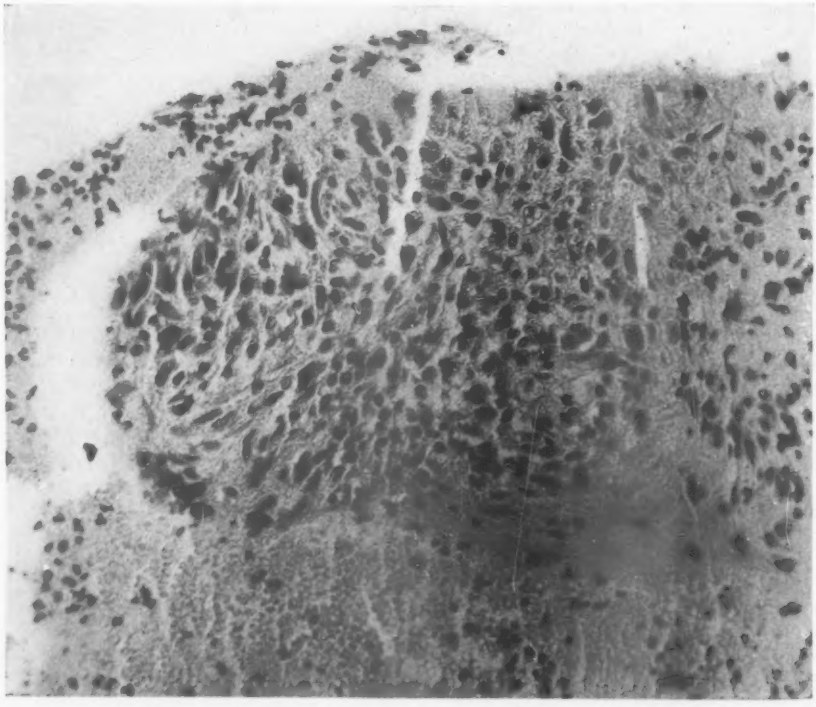
FIG. 6. Case 1. Cellular detail of Aschoff-like nodule. Hematoxylin and eosin stain. × 180.

FIG. 7. Case 2. Gross photograph of the mitral valve showing marked chronic rheumatic deformity. Subacute bacterial vegetations are indicated by arrow 2. A ruptured chorda tendinea, distant from this site of acute inflammation, is seen at arrow 1. It dangles from the free edge of the valve near the more recent vegetations, but is not involved in them.

FIG. 8. Case 3. Gross photograph of the aortic valve with old fibrotic distortion and a calcified vegetative mass toward the base of the non-coronary cusp, interpreted as representing a completely healed original focus of subacute bacterial endocarditis.



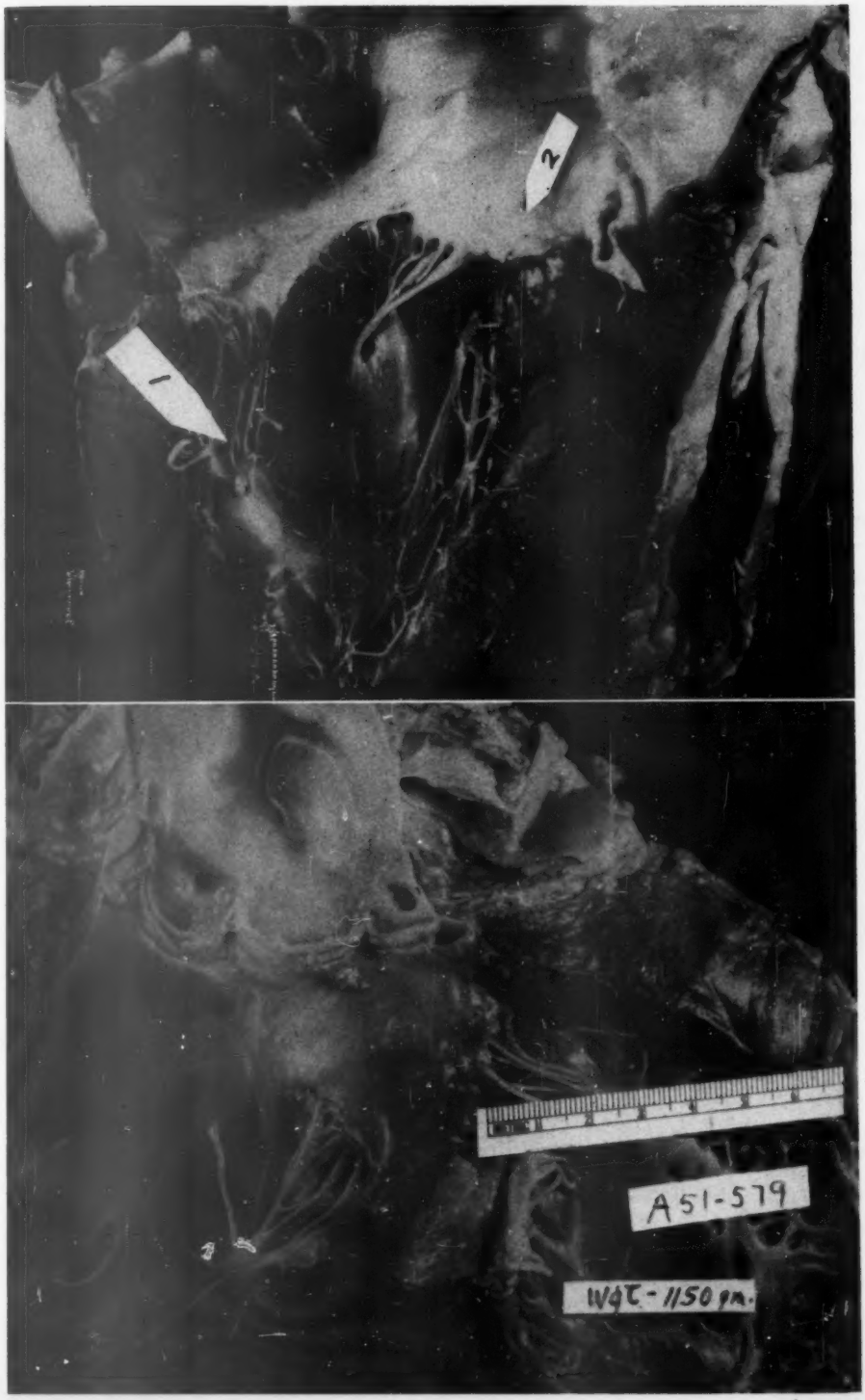


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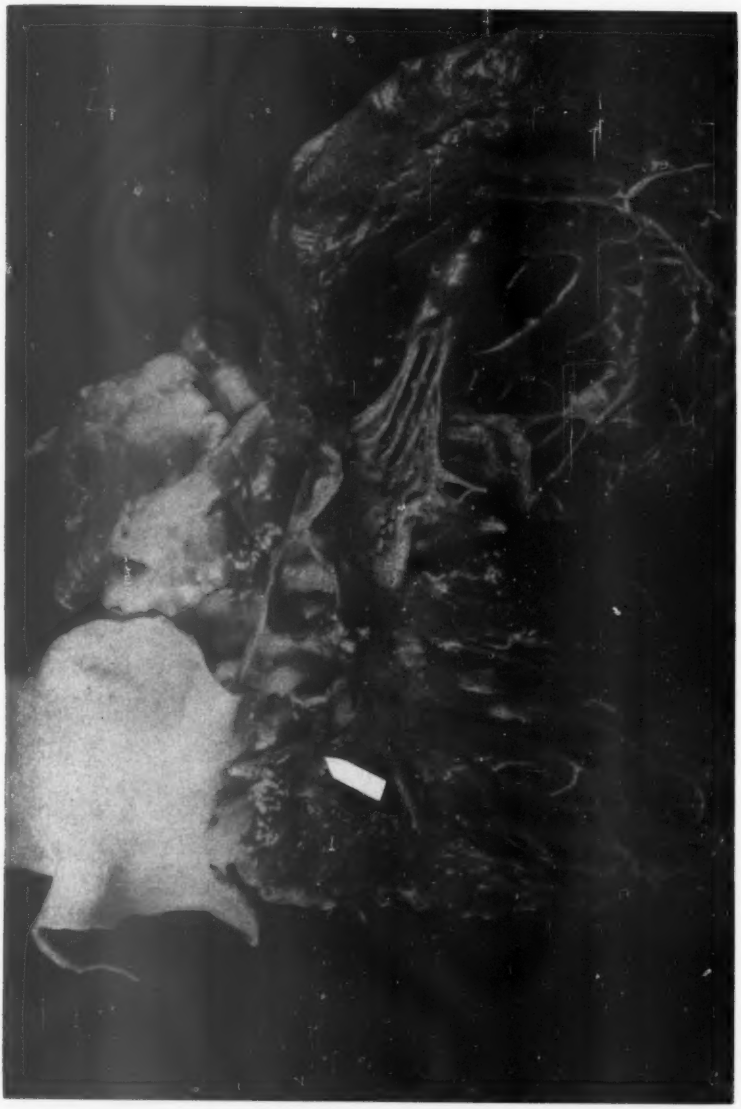
FIG. 9. Case 4. Gross photograph of the aortic valve with a subacute bacterial vegetation (indicated by the arrow), showing fibrosis and distortion of the adjacent valve. Of note are superimposed calcific vegetations on the aortic valve cusps and along the chordae tendineae of the mitral valve.



A MICROSPECTROPHOTOMETRIC STUDY OF THE DESOXYRIBOSE NUCLEIC ACID (DNA) CONTENT IN CELLS OF NORMAL AND MALIGNANT HUMAN TISSUES *

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Desoxyribose nucleic acid (DNA) is a constant and perhaps the most important nuclear constituent of all living cells. It is an essential part of chromatin and of the chromosomes, and is intimately concerned with the process of cell division. It would be reasonable to expect that the DNA content of tumor cells is different from that of normal cells. That such is the case is suggested by the increase in size and staining density of nuclei so frequently observed in tumors. To date, however, there are no chemical data to confirm or negate this concept.

This may seem surprising, but it can be explained by the lack of adequate chemical methods to attack such a problem. One must keep in mind that in order to answer the pertinent question whether the DNA content of a tumor cell is different from that of a normal cell, a special method is needed which permits the chemical analysis of a *single* cell. It is obvious that the conventional biochemical procedure which determines the DNA content in a mashed neoplastic tissue cannot give an answer because it yields only a value which refers to the fresh or dry weight of the tissue without discrimination between cellular and non-cellular material.

Even if biochemical analysis is done on a mass of isolated cells or nuclei (which in most tumors is nearly impossible because of the technical difficulties encountered in isolating nuclei of human tumors) the DNA content per cell is only an average value computed from the analysis of a mass of cells.[1] While such an average value may be representative for single cells in cell suspensions with a uniform DNA content, it is not significant for *the* single cell, nor does it reveal any variation from cell to cell if the suspension analyzed consists of cells with varying DNA content. The difficulties and pitfalls encountered in interpreting the biochemical results in suspensions of polyploid cells

* This investigation was supported (in part) by research grants C-1407 (C) and C-1814 from the National Institutes of Health, U.S. Public Health Service.

Presented in part at the Forty-third Annual Meeting of the American Association for Cancer Research, New York City, April 11, 1952, and in part at the Fiftieth Annual Meeting of the American Association of Pathologists and Bacteriologists, St. Louis, April 4, 1953.

Received for publication, June 1, 1953.

of liver have been demonstrated in previous studies[1] and discussed in detail.[2]

Fortunately enough, due to Caspersson's pioneering in 1936,[3] microspectrophotometric methods were developed which permit the quantitative estimation of chemical constituents of single cells or cell structures. By combining it with a photometric device, the microscope became a tool concerned with the chemistry of cells and tissues. The

TABLE I

Amount of DNA (Microspectrophotometry) in Cells of Normal Human Tissues

No. of cases	No. of nuclei measured	Range of ages	Sex	Tissue	Mean amount of DNA per nucleus in	
					Arbitrary units	Absolute amounts (10^{-9} mg.)
1	30	11 yrs.	F	Breast	3.25±0.09	6.50±0.18
1	30	51 yrs.	F	Cecum	2.58±0.05	5.16±0.10
1	30	62 yrs.	M	Kidney	3.17±0.04 5.43	6.34±0.08
5	140	3 mos. to 6 yrs.	M and F	Liver	2.82±0.08	5.64±0.16
8	150	13 to 86 yrs.	M and F	Liver	2.83±0.09 6.12±0.25 11.05±0.28	5.66±0.18 12.24±0.50 22.10±0.56
1	30	54 yrs.	M	Lung	3.02±0.07	6.04±0.14
1	30	65 yrs.	M	Lymphocytes	2.53±0.05	5.06±0.10
1	30	77 yrs.	F	Pancreas	2.59±0.17	5.18±0.34
4	168	1 to 68 yrs.	M and F	Skin	2.80±0.06	5.60±0.12
1	30	65 yrs.	M	Stomach	2.68±0.09	5.36±0.18
2	60	51 to 62 yrs.	M	Urinary bladder	3.12±0.15 5.68±0.23 9.80	6.24±0.30 11.36±0.46 19.60
21	630	23 to 45 yrs.	M	Spermatozoa	1.22±0.01	2.44±0.02

advantages are obvious; here for the first time, the appearance of a specific cell can be correlated directly *in situ* under the microscope with its chemical composition without destroying the cytologic and histologic architecture. Changes in structure, which of necessity must often be subjectively interpreted, can now be expressed in quantitative chemical terms.[4,5] Furthermore, the possibility of detecting quantitative changes in intracellular substances before the structural alterations in cells manifest themselves under the microscope (as for example, the decrease in the DNA content in sperm cells of infertile human males[6]) has not only opened completely new pathways for the study of disease, but shows also that the microspectrophotometric method may serve as a valuable diagnostic tool.

The purpose of the present study is an attempt to relate one of the most important chromosomal components, DNA, to the malignant transformation of cells. However, since very little was known about the DNA content of cells in normal human tissues prior to this report, a comparative extensive study on the DNA content of a variety of normal and malignant human tissues had to be carried out to establish a baseline.

For this study, results of the DNA measurements of nearly 2500

TABLE II

Amount of DNA (Microspectrophotometry) in Liver Cells of Normal Children and Adults

No. of cases	Ages	Sex	Cytologic appearance	No. of nuclei measured	Mean amount of DNA per nucleus in	
					Arbitrary units	Absolute amounts (10^{-9} mg.)
5	3 mos. to 6 yrs.	M and F	Normal, no mitosis	150	3.27±0.11	6.54±0.22
					2.70±0.06	5.40±0.12
					2.89±0.06	5.78±0.12
					2.41±0.12	4.82±0.24
					2.81±0.03	5.62±0.06
5	13 to 86 yrs.	M and F	Normal, no mitosis	150	2.65±0.09	5.30±0.18
					5.74±0.31	11.48±0.62
					11.69	23.38
					2.96±0.08	5.92±0.16
					6.26±0.37	12.52±0.74
					11.38	22.76
					3.45±0.21	6.90±0.42
					7.66	15.32
					2.31±0.09	4.62±0.18
					4.27±0.08	8.54±0.16
					9.20	18.40
					3.56±0.11	7.10±0.22
					7.09±0.23	14.18±0.46
					12.50	25.00

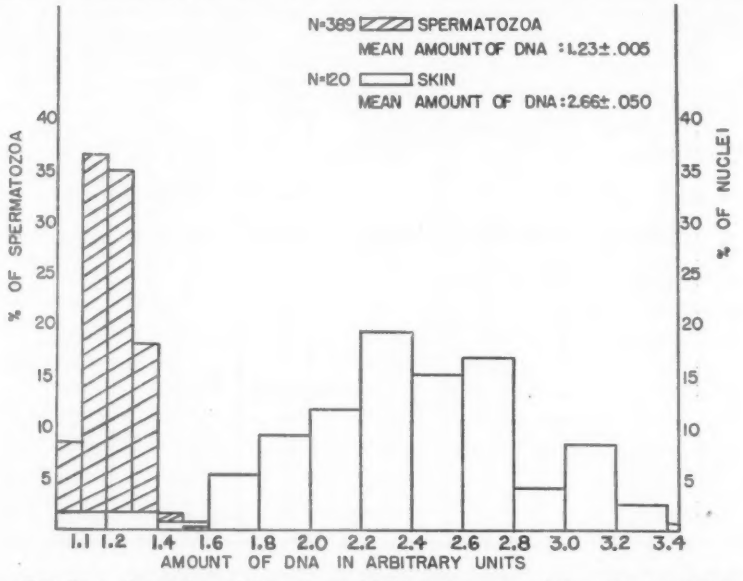
individual cells are presented, derived from 11 different tissues and from 76 individual cases. The validity of the quantitative microspectrophotometric methods in determining DNA in individual mammalian cells has been established in previous studies,[7] in the course of which it was found that the mean amounts of DNA in individual nuclei of beef tissues as determined by ultraviolet and Feulgen microspectrophotometry agreed closely with the results of DNA estimation done on the same cells by biochemical analysis.

MATERIAL AND METHODS

In order to carry out the investigation to be described, normal, precancerous, and cancerous tissues were secured from 76 human sub-

jects. Of these, 47 were normal, while 29 were patients with precancerous or malignant lesions. Whenever possible, surgical and biopsy material from Doctors Hospital, Cleveland, Ohio, was used. This was fixed, embedded, cut, and stained as previously described.[4] While, for some of the studies on normal tissues, post-mortem material had to be utilized,* it should be pointed out that due to the stable character of DNA, which does not change in amount for a considerable period after death, the results are not affected.

For the estimation of the amounts of DNA, the Feulgen reaction and microspectrophotometric analysis was used as previously de-



Text-fig. 1. Comparison of the amount of DNA (microspectrophotometry) in individual spermatozoa of fertile human males, and of individual normal human skin cells.

scribed.[4,8] All microspectrophotometric measurements were done on cells which appeared cytologically to be in interphase. For each tissue at least 30 individual nuclei were measured. The amounts of DNA per nucleus are expressed in arbitrary units as well as in absolute amounts. The former are given for the convenience of other workers in the field who use the same arbitrary units. The absolute amounts are based on previous studies[7,9] in which the biochemical analysis of DNA, when

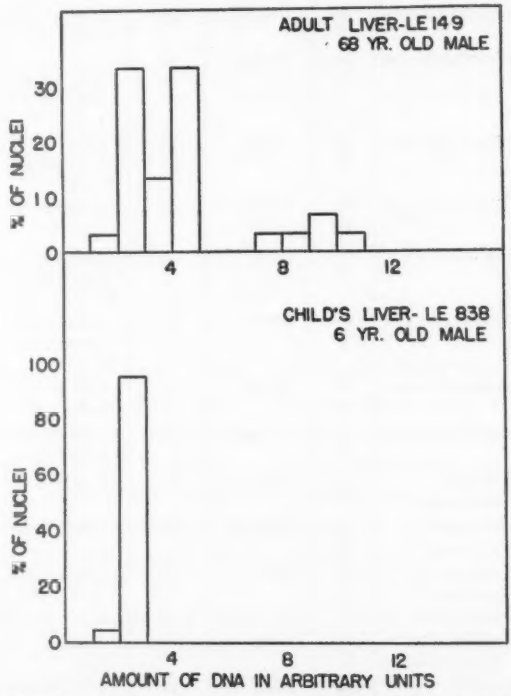
* We are indebted to Dr. Lester Adelson of the Institute of Pathology, Western Reserve University, and of the Cuyahoga County Coroner's Office for some of the post-mortem material used in this study.

compared with ultraviolet and Feulgen microspectrophotometric results, established that a factor of 2×10^{-9} mg. will convert arbitrary units to absolute amounts. In each case a statistical analysis was carried out and each mean value which was obtained on a number of nuclei larger than 10 is given with its standard error.

Results

The DNA Content of Cells of Various Normal Human Tissues

The results of the DNA measurements in 1358 individual nuclei of 11 different normal tissues in 47 individuals are presented in Table I.



Text-fig. 2. Individual DNA measurements (microspectrophotometry) in nuclei of liver in an adult and a child.

From this it is evident that there is a remarkable constancy of the DNA content in the somatic cells of the various human tissues examined. Each tissue contains cells with a basic mean DNA value of approximately 5.6×10^{-9} mg. (or approximately 2.8 arbitrary units) regardless of age, sex, or race of the individual. Furthermore, it can

TABLE III

Amount of DNA (Microspectrophotometry) in Nuclei of Precancerous Tissues and in Benign and Malignant Neoplastic Tissues

Case no.	Diagnosis	Age, sex	Tissue	No. of nuclei measured	Mean amount of DNA per nucleus in: Arbitrary units	Mean amount of DNA per nucleus in: Absolute amounts (10^{-9} mg.)
1 (Le 901)	Adenocarcinoma	22 F	Breast	30	3.69±0.08	7.38±0.16
2 (Le 902)	Adenocarcinoma	29 F	Breast	41	3.15±0.18	6.30±0.36
3 (Le 903)	Adenocarcinoma	33 F	Breast	30	3.09±0.10	6.18±0.20
4 (Le 904)	Adenocarcinoma	40 F	Breast	30	3.31±0.06 5.84±0.31	6.62±0.12 11.68±0.62
5 (Le 905)	Adenocarcinoma	49 F	Breast	30	3.75 6.64±0.17 9.73 14.55	7.50 13.28±0.34 19.46 29.10
6 (Le 906)	Adenocarcinoma	54 F	Breast	30	3.64 6.37±0.14 10.54	7.28 12.74±0.28 21.08
7 (Le 907)	Adenocarcinoma	59 F	Breast	30	4.06±0.16 8.30	8.12±0.32 16.60
8 (Le 908)	Adenocarcinoma	62 F	Breast	30	2.99±0.24 6.49±0.27 10.50	5.98±0.48 12.98±0.54 21.00
9 (Le 461)	Adenocarcinoma	67 F	Breast	40	2.78±0.07 7.00±0.27 12.06	5.56±0.14 14.00±0.54 24.12
10 (Le 909)	Adenocarcinoma	83 F	Breast	30	2.85±0.15 5.82	5.70±0.30 11.64
11 (Le 457A)	Adenocarcinoma	51 F	Cecum	38	3.03±0.15 6.70±0.19	6.06±0.30 13.40±0.38
12 (Le 428B)	Clear cell carcinoma	76 M	Kidney	55	5.21±0.16	10.42±0.32
13 (Le 852)	Adenoma of renal cortex	62 M	Kidney	30	2.80±0.07 5.97	5.60±0.14 11.94
14 (Le 450D)	Metastasis of pancreatic carcinoma	63 M	Liver	30	5.82±0.31	11.64±0.62
15 (Le 432)	Anaplastic bronchial carcinoma	69 M	Lung	28	3.13±0.15 6.45	6.26±0.30 12.90
16 (Le 916)	Partly differentiated adenocarcinoma	54 M	Lung	30	2.70±0.06 5.49±0.30 13.66	5.40±0.12 10.98±0.60 27.32
17 (Le 460A)	Metastasis of adenocarcinoma of stomach	65 M	Lymph node	30	4.40±0.34	8.80±0.68
18 (Le 452B)	Carcinoma	77 F	Pancreas (head)	40	2.43±0.11 6.03±0.19	4.86±0.22 12.06±0.38
19 (Le 450)	Carcinoma	63 M	Pancreas (head)	40	3.20±0.16 5.95±0.10	6.40±0.32 11.90±0.20
20 (Le 506)	Senile keratosis (Freudenthal)	67 F	Skin	48	5.3 ±0.27	10.6±0.54

TABLE III (Continued)

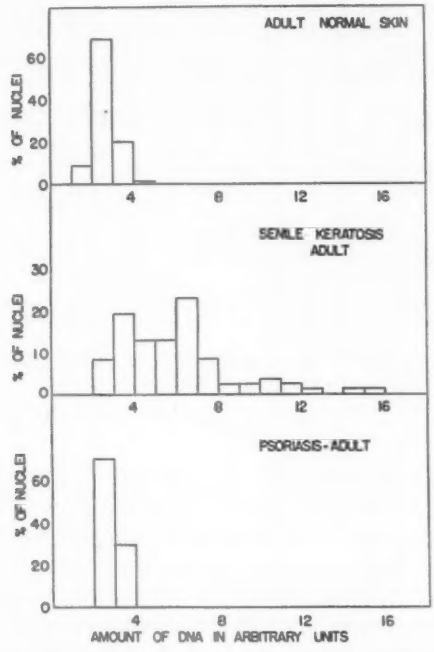
Case no.	Diagnosis	Age, sex	Tissue	No. of nuclei measured	Mean amount of DNA per nucleus in: Arbitrary units	Mean amount of DNA per nucleus in: Absolute amounts (10^{-9} mg.)
21 (Le 657)	Senile keratosis (Freudenthal)	68 M	Skin	35	6.60±0.60	13.20±1.20
22 (Le 634)	Senile keratosis (Freudenthal)	67 M	Skin	44	6.30±0.23	12.60±0.46
23 (Le 431B)	Diffusely growing undifferentiated carcinoma	66 M	Stomach	30	3.37±0.26 6.61±0.40	6.74±0.52 13.22±0.80
24 (Le 460B)	Adenocarcinoma	65 M	Stomach	30	3.43±0.21	6.86±0.42
25 (Le 426)	Seminoma	48 M	Testis	40	4.13±0.25 9.20	8.26±0.50 18.40
26 (Le 427)	Transitional cell carcinoma	51 M	Urinary bladder	30	6.13±0.51 12.55±0.49 17.68	12.26±1.02 25.10±0.98 35.36
27 (Le 467)	Diffusely growing transitional cell carcinoma	51 M	Urinary bladder	40	5.74±0.32 9.43±0.31 17.74	11.48±0.64 18.86±0.62 35.48
28 (Le 469)	Epidermoid carcinoma	62 M	Urinary bladder	58	3.72±0.15 6.62±0.21 11.07	7.44±0.30 13.24±0.42 22.14
29 (Le 917C)	Anaplastic glandular carcinoma of peri-urethral region (primary, ovary?)	66 F	Urinary bladder	30	3.95±0.20	7.90±0.40

be seen also that some of the tissues (for example: adult liver, kidney, urinary bladder) have, in addition, cells carrying amounts of DNA which are nearly exact multiples of the basic amount of DNA. The mean uniform basic DNA content in cells of human tissues with diverse metabolic activities and the occurrence of cells with multiple DNA values in some tissues are in full accordance with results on other animal tissues.[1,10-12]

Since previous comparative studies[8,9,13] established a direct relationship between counts of chromosomal numbers and DNA content of cells, it seems justified to consider the multiple amounts of DNA in some human tissues as an expression of multiple sets of chromosomes or, in other words, of polyploidy. A further confirmation of the relationship comes from the human sperm data[6] which, in accordance with their haploid chromosomal number, also show approximately half the DNA content found in the diploid somatic cells. Because the occurrence of multiple DNA classes (2DNA, 4DNA) was not observed in tissues of children, while they were always observed in the same tissues (*i.e.* liver) of adults, a study of the DNA content in normal livers

at different ages was made. A separate detailed report on the correlation of multiple DNA classes with age will appear elsewhere. A few examples pertinent to this study are presented in Table II. On the basis of the DNA data presented in this table, it can be seen that there is a definite relationship between the age of the individual and the occurrence of multiple DNA classes.

In the 5 children from 3 months to 6 years of age, only *one* DNA

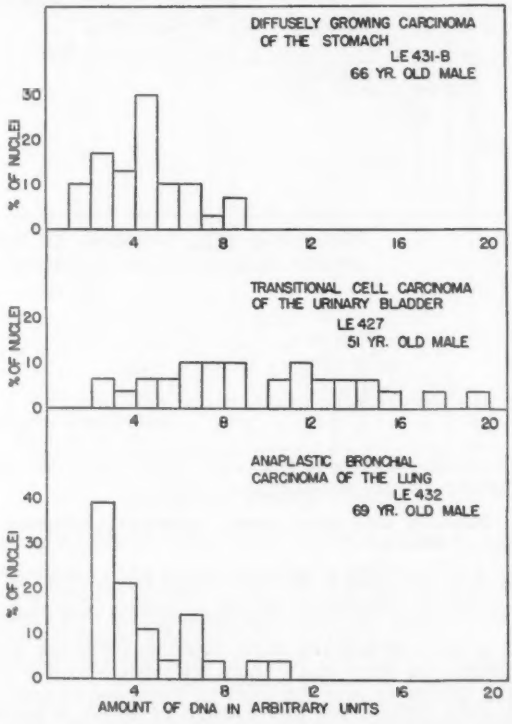


Text-fig. 3. Individual DNA measurements (microspectrophotometry) in nuclei of normal skin and in senile keratosis.

class, namely, the characteristic basic mean DNA value of approximately 5.6×10^{-9} mg., was observed while each of the 5 individuals from 13 to 86 years showed in addition one or two multiple DNA classes. It can also be noted from Table II for comparison, that only livers of children up to 6 years of age were selected. This was necessary because mitosis was encountered frequently in livers of children from 7 to 12 years. Since during mitosis the DNA content increases regularly in the interphase cells (as will be discussed later), demonstration of DNA classes is extremely difficult in mitotic tissues. Besides the

characteristic DNA content of each cell (1DNA ±, 2DNA ±; 4DNA ±), there is synthesized during mitosis an additional amount of DNA needed for the formation of the daughter cells. Since this quantity varies from cell to cell according to the stage of DNA synthesis (from 2DNA to 4DNA), a different amount is added to each cell which makes the establishment of DNA classes very complicated, if not impossible. Thus, for the comparison of DNA classes only non-mitotic tissues should be chosen.

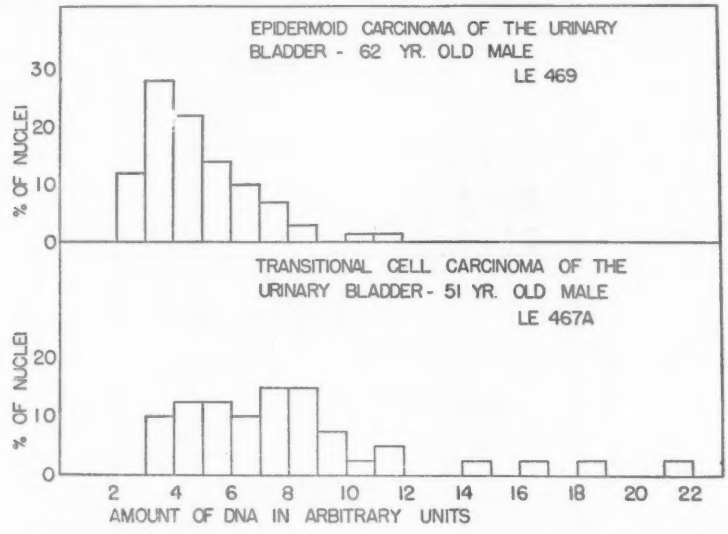
So far all DNA values on normal tissues have been given as *mean*



Text-fig. 4. Individual DNA measurements (microspectrophotometry) in nuclei of carcinomas in stomach, urinary bladder, and lung.

values per nucleus computed from the microspectrophotometric analyses of a number of individual cells. As pointed out repeatedly in previous studies,[1,2,9] such a mean DNA value, of necessity, gives no information as to the variation of the DNA content which may occur from cell to cell within the same tissue. In Text-figures 1 and 2 some

typical examples of the distribution of DNA values in individual cells of different normal tissues are presented. It is evident from the example shown (Text-fig. 1) that a great number of the cutaneous cells measured have DNA values which vary relatively little from each other and from the computed basic mean value, which lies between 2 and 3 arbitrary units as indicated by the peaks. However, it can be noted also that some of the cells have a DNA content which is significantly lower or higher than this basic value. A somewhat similar dis-



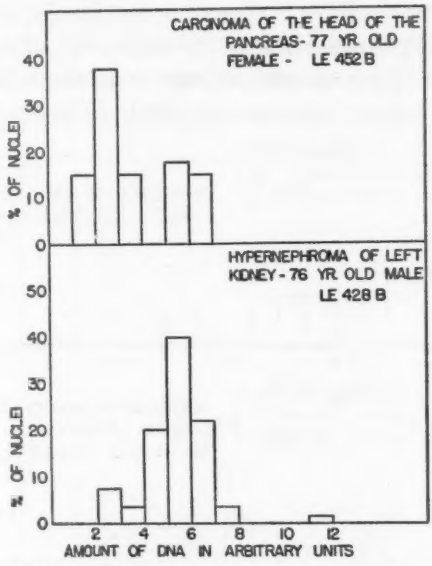
Text-fig. 5. Individual DNA measurements (microspectrophotometry) in nuclei of carcinomas of urinary bladder.

tribution curve is obtained if the individual DNA data of the haploid sperm cells are plotted. While in this instance the great majority of the spermatozoa contain nearly the same amount, there are also a certain number which have a deviating content of DNA.

The same holds true if the distribution of DNA is considered in a tissue with multiple amounts of DNA as shown in Text-figure 3. While the cells of the adult liver show definite peaks in their DNA amounts, between 2 and 3, 4 and 5, and 9 and 10, indicating polyploidy, somewhat smaller or larger DNA values may occur within each group. A very similar range of DNA values has been reported previously in animal and plant tissues and its possible biologic importance and relation to deviations in chromosomal numbers have been discussed.[1,2,7,9,14,15]

While it thus appears that the DNA content is not as constant in

each cell of a tissue as has been claimed by some workers,[11,12] the relative stability in the mean amount in cells of different tissues and of different individuals seems to be well established also for man (Tables I and II).



Text-fig. 6. Individual DNA measurements (microspectrophotometry) in nuclei of carcinomas in pancreas and kidney.

The DNA Content of Cells of Various Precancerous and Malignant Human Tissues

Using the relatively uniform mean DNA content in cells of normal tissues as a baseline, the question arises whether cells of precancerous or malignant tissues exhibit a similar constancy of DNA. The results of DNA measurements are presented in Table III.

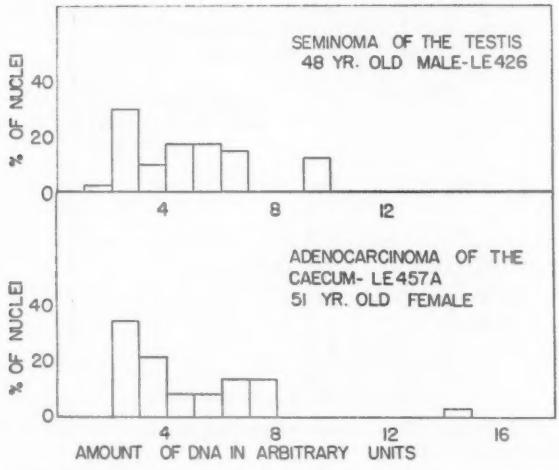
It can be seen from the data in Table III that the mean DNA values for the 29 tumors were not as constant and as uniform as for the 47 normal cases (Table I). These cases can be grouped into three categories:

The first group of tumors has DNA values which are either the same or only slightly higher than the basic value of the normal tissues (cases 2, 3, 4, 8, 9, 10, 11, 13, 15, 16, 18, 19, 23, and 24).

The second group has DNA values approximately 30 per cent higher than the basic normal DNA content (cases 1, 5, 6, 7, 17, 25, 28, and 29).

In the third group, cells with the basic DNA content are completely lacking and all the cells have as a lowest value a multiple (tetraploid) DNA content (cases 12, 14, 20, 21, 22, 26, and 27). The occurrence of such "tetraploid tumors" in humans is in conformity with tetraploid animal tumors.[9]

The differences between the DNA content of cells of normal and malignant tissues are even more obvious when the *individual data* for tumors and for normal cases are compared. As pointed out, the com-



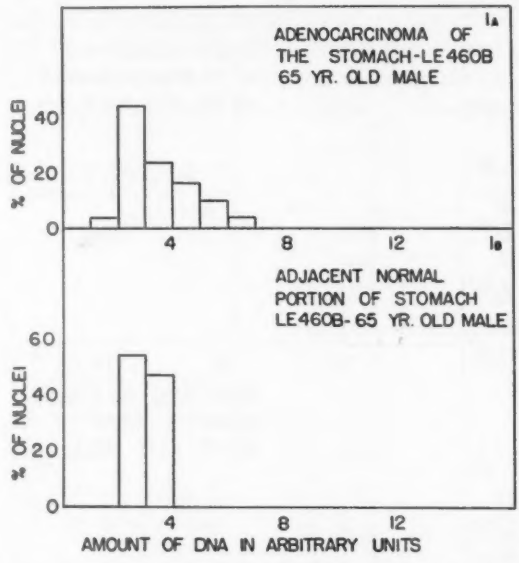
Text-fig. 7. Individual DNA measurements (microspectrophotometry) in nuclei of a seminoma of testis and carcinoma of cecum.

puted mean DNA value does not allow an appraisal of the variability of cells within a tissue.

In Text-figures 3 to 10, typical examples of the DNA distribution in individual cells of various types of tumors are presented. It can be seen that all of the primary tumors (and the so-called precancerous stages of senile keratosis) as well as the metastases have cells which show a marked variability of the DNA content. This spread of the DNA data and the frequent occurrence of DNA values intermediate between the multiple DNA classes is especially striking when compared with the relatively small variability of the DNA values in normal homologous cells (Text-figs. 8, 9, and 10). It can be noted also that the spread of the DNA data of metastatic nodules is even more extensive than that of the primary tumor.

The unequal amounts of DNA in the cells of malignant tissues have

been found so far in every tumor examined. However, since the tumors studied fall within a limited age group (namely, 48 to 77 years), the question arises whether in younger individuals the tumors show a similar variation of DNA in their cells. Such an investigation seems pertinent because, as pointed out previously, normal tissues (*i.e.*, liver) of young persons have cells with only one DNA class while the same normal tissue of older persons always has two or more DNA classes.



Text-fig. 8. Individual DNA measurements (microspectrophotometry) in nuclei of an adenocarcinoma of stomach and of adjacent normal portion of stomach.

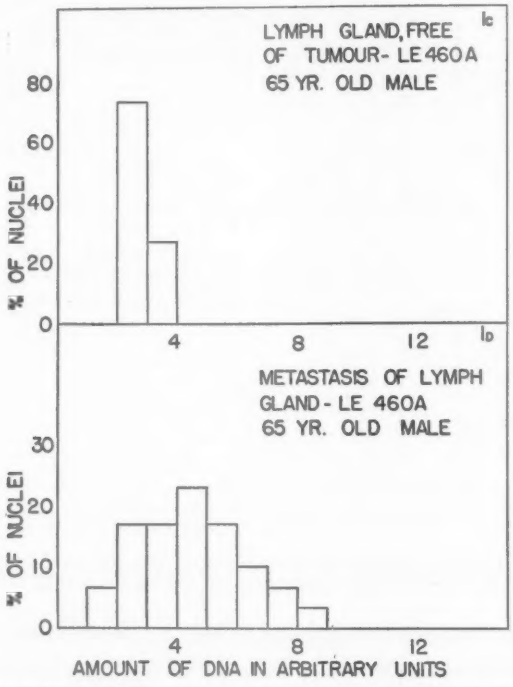
In order to examine the question, adenocarcinoma of the breast was chosen because it represents a type of tumor which occurs in a particularly wide range as to age. DNA was studied in 9 women from 22 to 83 years of age. The results of the individual DNA measurements are given in Text-figures 11 to 14. On the basis of the data obtained so far, it seemed that the breast tumors of the younger age group (22 to 33 years) show less variability from cell to cell and a lower DNA content than those of the older age group. All 6 cases between 40 and 83 years exhibited a more pronounced variation from cell to cell and higher DNA contents than the 3 cases of the younger age group. It is realized, of course, that a much larger number of cases is necessary before this relationship can be considered as established.

Discussion

On the basis of the preceding studies the findings may be summarized as follows:

Results for Normal Tissues

All normal tissues examined so far showed a very similar basic mean DNA value in the nuclei of their cells. This DNA content was approximately twice that of the sperm cell and was probably characteristic for cells with diploid chromosomal numbers.



Text-fig. 9. Individual DNA measurements (microspectrophotometry) in nuclei in a metastatic nodule in a lymph node from the adenocarcinoma of stomach (Text-fig. 8) and in an adjacent normal lymph node.

Some of the normal tissues carried cells with nearly exact multiples of the diploid DNA content, that is, tetraploid and octoploid amounts. In spite of the similarity of the mean DNA value in the different normal tissues, there was a certain degree of variation in the amount of DNA from cell to cell within each tissue.

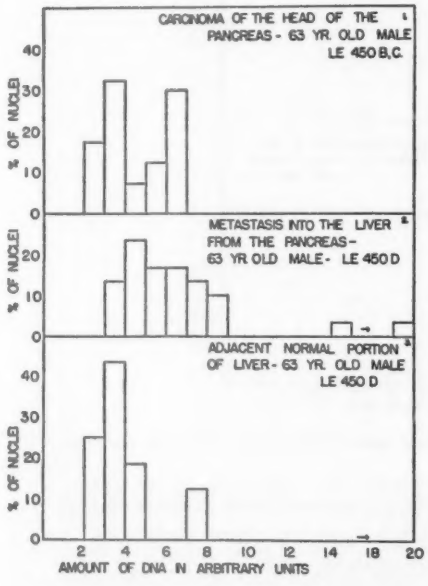
Results for Malignant Tissues

In contrast to the presence of a basic mean DNA content in *all* nor-

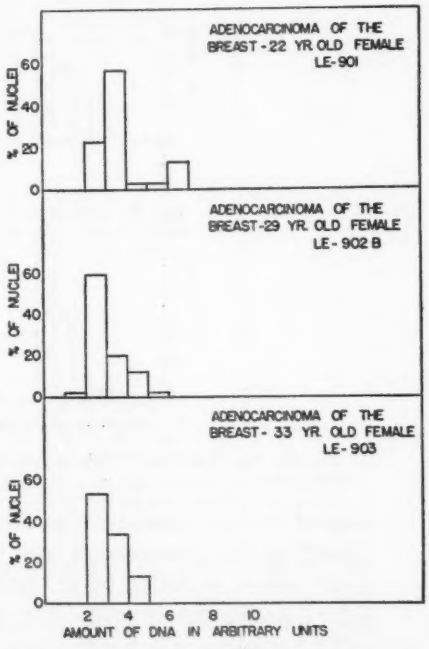
mal tissues, precancerous and malignant tissues often deviated from this basic amount. In such cases DNA values were either considerably higher (30 per cent) than the basic value, or showed as their lowest amount the double value.

In contrast to the relatively small variability of DNA from cell to cell within a normal tissue, *all* malignant cells showed a much wider fluctuation in their DNA content from cell to cell.

The findings of the relative stability and constancy of the DNA content in cells of a variety of normal human tissues and of different individuals is in good agreement with the observations on animal tissues. This constancy of the basic DNA content is indeed striking, espe-



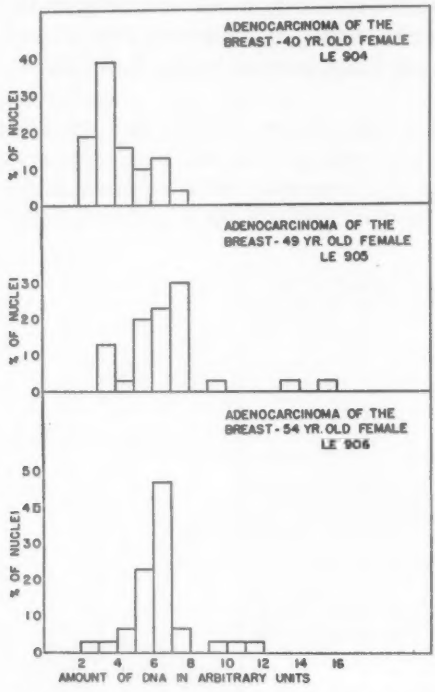
Text-fig. 10. Individual DNA measurements (microspectrophotometry) in nuclei of a carcinoma of pancreas, of a metastatic nodule of this carcinoma in liver, and of the normal liver.



Text-fig. 11. Individual DNA measurements (microspectrophotometry) in nuclei of adenocarcinoma of breast, ages 22 to 33 years.

cially if considered in the light of the diverse metabolic processes going on in the cells of the different tissues. However, if one takes into account the present-day concept that DNA is an integral part of only the chromosomes and that the chromosomal numbers, up to a certain degree, are more or less constant for a cell, then this constancy is not

too surprising. This apparent correlation between chromosomal number or mass and DNA content is further supported by the parallelism between chromosomal counts and DNA content, as, for example, in haploid germ cells[6] or tetraploid ascites tumors.[9] It thus appears that



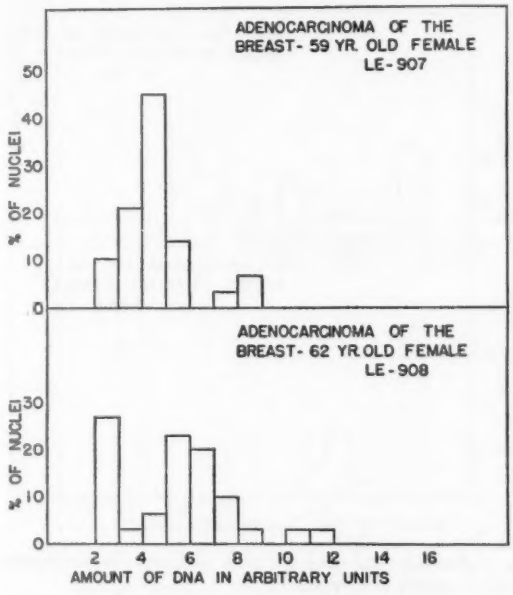
Text-fig. 12. Individual DNA measurements (microspectrophotometry) in nuclei of adenocarcinoma of breast, ages 40 to 54 years.

cells of normal tissues have an orderly pattern of DNA content linked closely to the chromosomes and, though there are some variations in some cells as indicated by the individual data, most of the tissue cells have a similar characteristic average amount.

This stability, which indicates a certain static behavior of the cell in regard to its chromosomal constituents, is actually to be expected in cells of a non-growing normal tissue. On the other hand, the marked fluctuation of DNA observed in the neoplastic tissue again indicates the close linkage of DNA to the chromosomal behavior. The characteristic feature of each tumor, namely, the formation of new cells and the occurrence of mitotic figures, *must* of necessity lead to changes in

the DNA contents. The scatter of the DNA data is just an indication of the DNA synthesis necessary for the formation of new cells.

Thus it appears that the deviation in DNA data in the tumors can be explained simply on the basis of growth and mitotic processes alone, without even considering the *malignant* character of the cells. This concept is well supported if one looks at the DNA data of cells of a *normal* mitotic human tissue. While mitosis is rarely encountered in adult human liver, we have observed mitosis in livers of children from

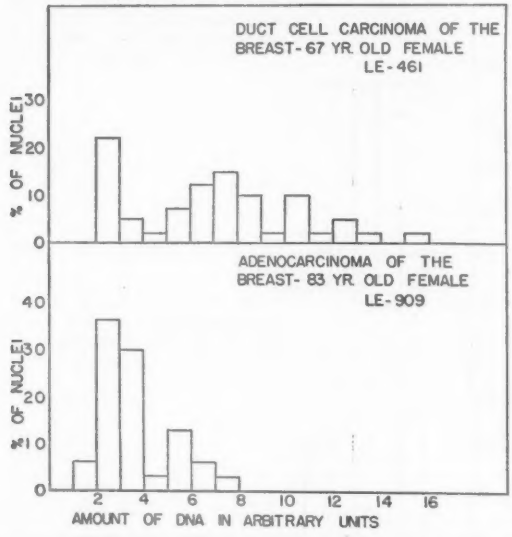


Text-fig. 13. Individual DNA measurements (microspectrophotometry) in nuclei of adenocarcinoma of breast, ages 59 to 62 years.

9 to 12 years. It is evident from the DNA data in Text-figure 15 that interphase cells of a normal liver undergoing mitosis also show a variability and increase in the DNA content as compared with the cells of a liver which is not in the process of mitosis (see Text-fig. 2, case Le 838). The picture is obviously similar to that of malignant cells (perhaps somewhat less striking than in some tumors); but the resemblance is even closer, if one considers that in both instances it is the so-called interphase or resting cell which displays the increase in DNA. Apparently also in human tissues (regardless of whether they are normal or malignant), the build-up of DNA to its double value takes

place at a very early stage of mitosis, that is, *before* structural changes in regard to the chromosomes can be visualized. These findings are in good accord with the ones obtained in dividing animal and plant tissues.[2,12,14,16-18]

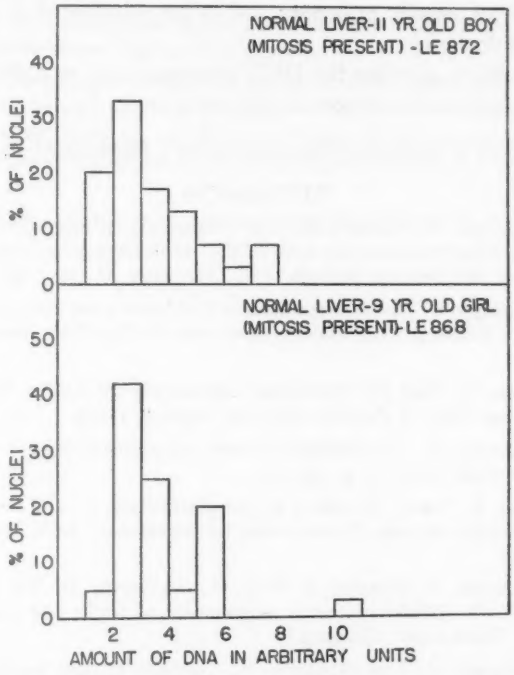
The possibility of interpreting deviating DNA data in tumors only on the basis of mitosis may be a disappointment to the pathologist who has tried again and again to find a diagnostic tool for the recognition of malignant cells. However, it must be kept in mind that normal tis-



Text-fig. 14. Individual DNA measurements (microspectrophotometry) in nuclei of carcinomas of breast, ages 67 to 83 years.

sues in adults usually do not exhibit mitosis, or in other words they usually show the characteristic DNA value with relatively little scattering. Consequently, a DNA histogram of a tissue which reveals scatter and increase of DNA must be looked upon with suspicion in regard to malignancy, unless regeneration is to be expected. One may argue, of course, that microscopic examination of a tissue for mitosis is much simpler and will lead to the same results. However, the absence of mitotic figures in a tissue does not exclude the possibility that a mitotic process is going on. If the time period of the mitotic cycle is a very rapid one, mitotic figures may be missed completely. Since, as pointed out before, DNA synthesis occurs at a very early stage of the mitotic process, namely, already in interphase, and is thus independent of the

time period of the mitotic cycle, DNA increase can be used as a most sensitive indicator for a division process. Studies of this kind may be particularly helpful in the cases which the pathologist designates as borderline cases, such as carcinoma *in situ*, some cases of low-grade malignancy, and, in brief, cases in which mitotic figures are scanty or absent.



Text-fig. 15. Individual DNA measurements (microspectrophotometry) in nuclei of livers of 2 children (mitosis present).

Summary

Microspectrophotometric studies of the DNA content in approximately 2500 individual cells of 49 normal and 27 malignant tissues of humans (ages ranging from 3 weeks to 87 years) gave the following results:

All normal tissues contained cells with a similar basic mean DNA content of 5.6×10^{-9} mg. (or 2.8 arbitrary units). Some tissues (*i.e.*, adult liver) in addition carry cells with nearly exact multiple amounts.

Within normal tissues the DNA content shows a certain but limited degree of variation from cell to cell.

Precancerous and malignant tissues do not exhibit the same uniformity in their mean DNA content as the cells of the homologous normal tissues.

Malignant tissues also reveal a much larger scatter from cell to cell than do the normal cells.

The deviating behavior of DNA in malignant tissues is interpreted on the basis of mitotic processes and is not considered a specific criterion for malignancy.

The possibility of using the DNA measurements as a diagnostic aid in certain questionable tumors is discussed.

It is a pleasure to thank Miss Ethel Lieb for her valuable assistance.

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FAMILIAL OCCURRENCE OF "IDIOPATHIC" CALCIFICATION OF CEREBRAL CAPILLARIES *

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In his review of the literature in 1948, Löwenthal[1] found 32 cases of idiopathic non-arteriosclerotic intracerebral vascular calcification, or Fahr's disease, among which were 3 instances of familial occurrence. These cases were proved by x-ray demonstration of cerebral calcification, biopsy, or necropsy. The following 2 instances of this condition, one occurring in a white male child who died at the age of 33 months, and the other in a younger brother who died at 31 months, are reported because of the rare familial occurrence.

REPORT OF CASES

Family History. On the maternal side a half-brother died of unknown cause at the age of 2 years, death being ascribed either to diarrhea or pneumonia. Two sisters of the maternal grandmother each had one stillborn male infant. The mother was well at 24 years of age when the history was taken. In addition to the 2 children affected with this disease, she had had one spontaneous abortion.

On the paternal side, there were no significant data.

Case 1

A white male child was admitted to Tripler General Hospital at the age of 6 months because of convulsions of recent development. The pregnancy and birth were uneventful. The birth weight was 7 lbs. 3 oz. Shortly after birth, there developed alternating constipation and diarrhea, poor eating, and frequent episodes of vomiting. For several weeks prior to admission there was a nasal discharge associated with fever.

Physical Examination. On admission the child was malnourished, dehydrated, and weighed 9 lbs. 2 oz. The temperature was 102° F. There were enlarged anterior cervical lymph nodes, but no other positive physical findings.

Laboratory Studies. The urine contained a trace of albumin; the red blood cell count was 3.78 millions; hemoglobin, 12 gm.; white blood cells, 10,300, and the differential count was within normal limits. A stool examination was positive for fat and negative for undigested food and starch. The level of the blood cholesterol was 365 mg. per cent; the fasting blood sugar, 87 mg. per cent. Roentgenograms of the skull, long bones, chest, and gastro-intestinal tract were all within normal limits. An electro-encephalogram was interpreted as normal.

* The opinions or assertions contained herein are those of the author and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

Received for publication, June 7, 1953.

Course. Supportive treatment was instituted but with little improvement. Skull trephinations were performed bilaterally without giving evidence of subdural hematoma or other lesions. The child was discharged with a diagnosis of primary mental deficiency. At the age of 15 months he was re-admitted to this hospital with similar complaints, but repetition of the previous studies again was not diagnostic. At the age of 2 years and 10 months the child was examined in the Out-Patient Department of the U.S. Naval Hospital, Oakland. At that time he gave evidence of further mental and physical retardation, the level of development being considered at less than 2 years. His diet, which had always been poor, now consisted mostly of milk. There had been fever and vomiting 6 days prior to admission; he had then developed right-sided paralysis.

On admission his weight was 21 lbs. He had a tendency to hold his head to the left. His left arm was spastic. He was unable to move the right arm and leg, yet the deep tendon reflexes were active and equal and there were no pathologic reflexes.

Laboratory Studies. The serum sodium was 135 m.eq. per liter; calcium, 11.4 mg. per cent; potassium, 4.1 m.eq. per liter. The serum cholesterol was 330 mg. per cent. Phosphorus was 7.04 mg. per cent and chlorides 634 mg. per cent. Repeated examinations of the urine revealed 4 to 8 red blood cells with 10 granular casts per high-power field. The total serum protein was 6.3 gm., albumin being 2 gm. and globulin 4.3 gm. The white blood cell count was 18,000 with a normal differential. A blood culture was reported as negative. The spinal fluid contained 6 red blood cells and 3 white blood cells per high-power field. The protein in the spinal fluid was 38.4 mg. per cent; sugar, 83 mg. per cent; chlorides, 880 mg. per cent; Pandy's test, negative. Roentgenograms of the skull and other bones again were negative. The hospital stay of approximately 1 month was characterized by episodes of fever, increased spasticity, and finally coma terminating in death.

Necropsy Findings

Gross Examination. At necropsy the brain weighed 767 gm. It was obviously reduced in size, but showed, externally, only diffuse swelling and engorgement of the pial vessels. Coronal sections demonstrated enlargement of the lateral ventricles which measured 18 mm. in width at the level of the anterior horns. The most conspicuous gross change was the presence of symmetric areas of softening and hyperemia, having a granular or sandy consistency. These were located in the centrum semi-ovale of the frontal and parietal white matter (Figs. 1 and 2) and in the superior aspect of the lenticular nuclei. In the white matter of the parietal and occipital lobes there were no areas of softening, but a similar granularity was palpated. There were no changes in the brain stem, but granular areas were noted also in the white matter of the cerebellum.

Other significant findings at necropsy were: atrophy of the testes, thyroid gland, and thymus. Each testis weighed 3 gm.; the thyroid gland, 2 gm.; and the thymus, 5 gm. There was also an incomplete congenital stricture of the lower third of the right ureter with a mild right hydro-ureter and hydronephrosis. A Meckel's diverticulum,

measuring 3.5 cm. in length and 1 cm. in diameter, was present 115 cm. proximal to the ileocecal valve.

Microscopic Observations. Sections of the brain revealed a striking picture of calcification of the capillaries and, to a lesser extent, of the arterioles and venules in both the cerebral gray and white matter. The changes occurred in foci, predominantly in the central white matter (Fig. 3), but also in segments of the cortex lying in troughs of the sulci; in the latter, only the deep layers were involved. A similar change was present in the various parts of the thalamus (Fig. 4), but particularly in its lateral nucleus, in the major part of the putamen (Fig. 5), and to a lesser extent in the globus pallidus. The mildest change was noted in the dentate nucleus (Fig. 6) and adjacent white matter of the cerebellum. In all of these areas the capillary bed was prominent by virtue of deposition of a lime-like substance, varying from discrete droplets to homogeneous fused masses. This substance stained blue-black with hematoxylin and eosin, black with von Kossa's method, red with iron-carmine, and deep blue with Holzer's crystal violet[1a] method. These histochemical reactions suggested that the deposited material was calcium rather than iron or amyloid. Variable changes were noted in the underlying tissues, such as gliosis by astrocytes and their fibers, and areas of necrosis with fat-containing gitter cells. These changes, however, were mild to moderate and became apparent only where the calcification was most pronounced, suggesting that they were secondary to the vascular disorder.

Other pathologic findings included: Amyloid deposits in the arterioles of the splenic corpuscles; mild hyperplasia of the parathyroid glands, which were composed mostly of principal cells, a rare oxyphilic cell, and an occasional uninucleated or multinucleated giant cell; degeneration of the convoluted tubular epithelium of the kidneys with droplet formation, necrosis, and many hyaline casts, particularly in the collecting tubules and most striking in the right kidney. The thyroid gland was composed mostly of follicles of small to moderate size which were lined with columnar epithelium and had a poor colloid content. The pituitary gland was not remarkable.

Comment

This case is a classical example of so-called idiopathic calcification of the cerebral vessels. The lesions were characteristically symmetric and involved predominantly the central white matter of the frontal lobes, the basal ganglia, the thalamus, and dentate nuclei of the cere-

bellum. There was no clinical or chemical evidence of parathyroid deficiency and histologically the parathyroid glands showed a mild hyperplasia. The nephrosis probably had been present for some time since there was mild albuminuria at the age of 6 months and granular casts later, persistently high normal blood cholesterol, and a reversal of the albumin-globulin ratio. The amyloidosis of the spleen probably was secondary to the nephrosis. Other findings were underdevelopment of the tnymus, thyroid gland, and testes, and congenital anomalies in the form of partial stricture of the right ureter, a Meckel's diverticulum, and an accessory spleen.

Case 2

The younger brother of the patient designated as case 1 was admitted to Tripler General Hospital at the age of 2 months with complaints of constipation, fever, and vomiting. The child weighed 6 lbs. 13 oz. at birth. During pregnancy the mother had mild bleeding during the first 3 months but continued to a full-term, spontaneous delivery. The child did not breathe spontaneously immediately after birth and considerable assistance was necessary to start respiration. Bouts of fever with elevation of temperature to 101° and 120° F. had been present daily for 1 week. The vomiting had become more forceful 3 days prior to admission.

Physical Examination. On admission the child was pale, poorly developed, and weighed 7 lbs. 12 oz. There was moderate dehydration and poor turgor of the skin. There were no other physical abnormalities.

Laboratory Studies. The red blood cell count initially was 2.8 millions but with treatment rose to 4.57 millions. The differential count was 30 per cent neutrophils and 70 per cent lymphocytes. Urinalysis was normal; the serum sodium was elevated to 329 mg. per cent. The spinal fluid was normal, showing a total protein of 24 mg. per cent; sugar, 62 mg. per cent; no cells; a normal colloidal gold curve; and a negative Wassermann test. Studies for toxoplasmosis were negative. Adrenal insufficiency was considered, but the addition of sodium chloride to the feeding formula produced no clinical improvement. Dural taps at the age of 2½ months revealed no evidence of subdural hematoma. A pneumo-encephalogram done at the age of 3½ months was interpreted by one observer as showing "left cerebral atrophy and a small cyst of the 5th and 6th ventricles," and by another observer, as being within normal limits.

Course. The further course was characterized by repeated vomiting, constipation, and fever. At the age of 4½ months, the infant weighed 8 lbs. 6 oz., and he was discharged from the hospital with a diagnosis of mental deficiency, cause undetermined.

The patient was first seen at the U.S. Naval Hospital, Oakland, California, at the age of 11 months with complaints of poor gain in weight, poor eating, constipation, and intermittent convulsions. His physical development was delayed, as evidenced by the fact that he could not hold his head up until the age of 6 months and at the age of 11 months (on admission) he could not sit up. He had four teeth. Fever had been almost constant. At that time the child weighed 9 lbs. 2½ oz. He was poorly nourished but seemed happy and followed objects with his eyes. The circumference of the head was 16 inches; that of the chest, 14 inches; and the anterior fontanelle was 3 by 2 cm. There was a slight discharge from the nose and a mild congestion of the tonsils and pharynx. The abdomen was soft and doughy. The heart and lungs

were negative. The testicles were descended. Neurologic examination revealed hyperactive deep tendon reflexes.

Laboratory Studies. Non-protein nitrogen was 83 mg. per cent; creatinine, 0.625 mg. per cent; sodium, 161 m.eq. per liter; and potassium, 5.0 m.eq. per liter. A complete blood count was negative except for mild anemia. Urinalysis was negative. Roentgenograms, including a skull plate, were negative. Subdural taps were performed but were unrevealing. Intravenous pyelograms showed poor concentration of the dye. The patient was discharged 1 month after admission.

The third admission to the same hospital was at the age of 12½ months. The child then weighed 9 lbs. 10 oz. There had been no change in the clinical course. The serum sodium remained at the high level of 165 m.eq. per liter. The blood calcium levels were normal. Chlorides were 114 m.eq. per liter; cholesterol, 243 mg. per cent; non-protein nitrogen, 42 mg. per cent. Spinal fluid sugar was 55 mg. per cent and protein, 38 mg. per cent.

At 2 years of age the child suffered an ear and throat infection with a fever of 105° F. He was examined and treated elsewhere and his weight was then 9 lbs. Since that time he had improved considerably in nutritional status so that at 2½ years of age his weight was 16 lbs. He could also stand but was unable to walk.

He was admitted to another activity at the age of 31 months with a history of fever of 104° F., and episodes of convulsions for 3 days. Physical examination was negative and the following day he improved considerably. On the third hospital day there was a recurrence of the fever to 102° F. with onset of tonic convulsions and generalized rigidity which was more marked on the right side. He developed cyanosis, tachycardia, and slow, deep, regular respirations. The remaining physical examination at that time was negative. There was progressive coma with death occurring on the fourth hospital day.

Laboratory Studies. The urine was alkaline, with negative albumin and 2 to 4 white blood cells per high-power field. Spinal fluid was clear, with sugar 50.6 mg. per cent; chlorides, 734 mg. per cent. No organisms or cells were seen on microscopic examination. The white blood cell count was 5,850 with 85 per cent segmented cells and 15 per cent lymphocytes. Red blood cell count was 3.84 millions with hemoglobin of 10.5 gm.

Comment

The clinical course of this child closely resembled that of his older brother. In both cases there were mental and physical retardation, persistent febrile episodes, constipation, convulsive seizures, and laboratory evidence of high normal serum cholesterol. In case 1 there was proved nephrosis while in case 2 there was evidence of renal disease as revealed by poor concentration of the dye for the pyelogram. In both cases roentgenograms of the head failed to demonstrate cerebral calcification.

Necropsy Findings

The brain, in its fresh state, weighed 1000 gm. and showed diffuse symmetric swelling. Sections prepared from limited material available showed scattered calcareous deposits in capillaries, particularly in the subcortical white matter (Fig. 7) and in the putamen and globus pallidus (Fig. 8). Sections from the spinal cord, pons, and cerebellum showed no such deposits. There were no parenchymal changes.

Discussion

In reviewing the literature, it is apparent that the condition of "idiopathic" calcification of cerebral capillaries is not a disease entity. It can occur at different age periods. In the adult form it usually presents a picture of organic psychosis,[2] often with extrapyramidal signs[3] while in the infantile form it frequently manifests itself as a convulsive disorder. As stated before, of the 32 cases summarized by Löwenthal,[1] there were only 3 familial instances.

In recent years, Eaton[4,5] and his associates have presented clinical and chemical evidence of parathyroid deficiency in some of these cases and, moreover, favorable response to therapy with calcium and parathormone has been stressed in such instances. However, in the 2 cases reported here there was no clinical evidence to suggest hypoparathyroidism. Furthermore, in case 1, examination of the parathyroid glands showed no demonstrable pathologic condition. This would be in keeping with the consideration that some cases of Fahr's disease are probably due to other, and perhaps local, disturbances of metabolism rather than to a general disturbance in blood calcium brought about by parathyroid insufficiency. The familial occurrence in these cases would suggest inheritance of a specific metabolic difficulty similar to the familial occurrence of, for example, lipid metabolic disorders.

Summary

The rare familial occurrence in two male siblings of non-arteriosclerotic idiopathic calcification of the cerebral vessels, or Fahr's disease, is reported. The complete findings at necropsy are included in the case report of the older brother who died at the age of 33 months, plus the findings in the brain of the younger brother who died at the age of 31 months.

It is my opinion that this condition is not a disease entity. Although some cases appear to be due to hypoparathyroidism,[5] others may be on the basis of local disturbances of calcium metabolism. Still others, as in the cases presented here, appear to be the result of an inheritance of a specific metabolic disorder.

I am indebted to Dr. Nathan Malamud, Neuropathologist at the Langley Porter Clinic, San Francisco, California, for his assistance in preparing this paper.

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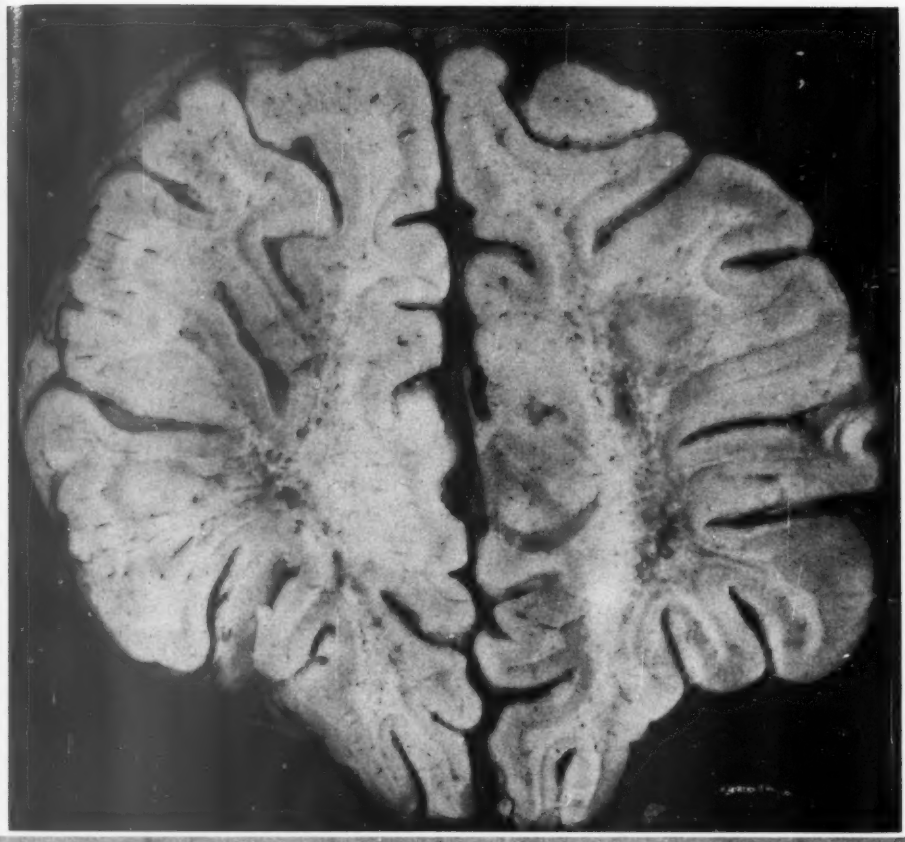
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Case 1. Coronal section of frontal lobes, showing symmetric areas of granular softening in the centrum semi-ovale.

FIG. 2. Case 1. Enlarged view of the gross appearance of the right centrum semiovale as shown in Figure 1. Small beads of calcium stand out in the vascular areas. × 5.

1



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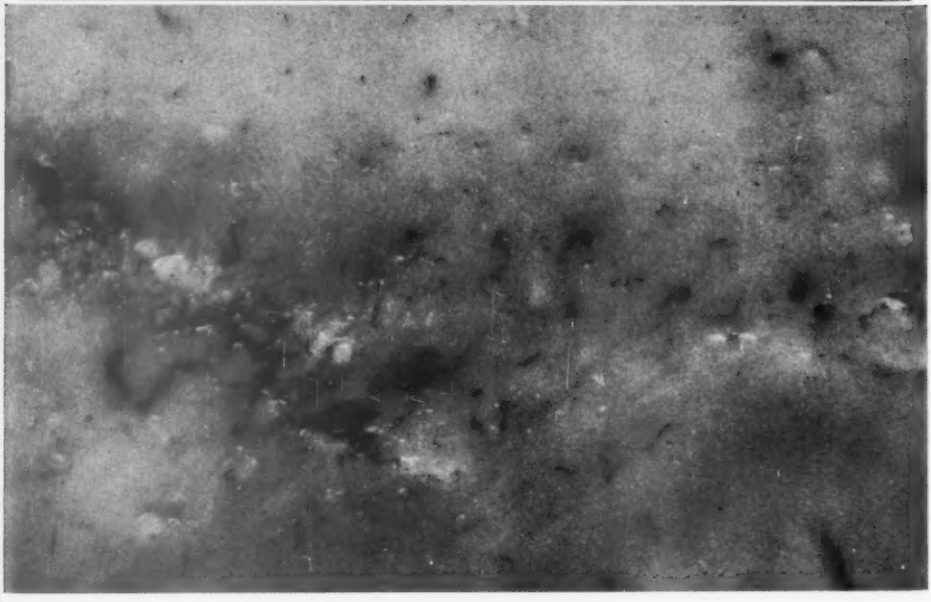


FIG. 3. Case 1. Cerebral (frontal) white matter, deep layers of cortex, showing striking calcification of the capillary bed, with deposition varying from discrete droplets to homogeneous fused masses. Von Kossa's method for calcium. × 100.

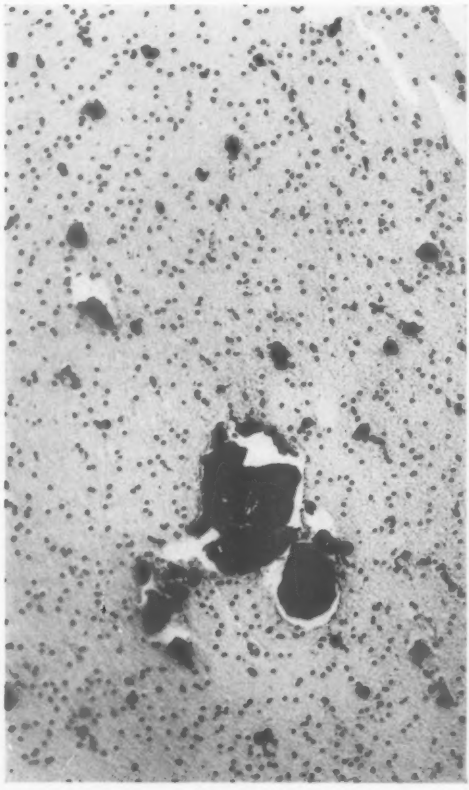
FIG. 4. Case 1. Thalamus. A change similar to that in Figure 3 is present. Cresyl violet (Nissl) stain. × 100.

FIG. 5. Case 1. Putamen and globus pallidus. Abundant calcification. Cresyl violet (Nissl) stain. × 40.

FIG. 6. Case 1. Dentate nucleus. Calcification is present although less marked than in the region illustrated in Figure 5. Weil's myelin stain. × 100.

FIG. 7. Case 2. High power view of area of the cerebral cortex, showing fused masses of calcium within small capillaries. Hematoxylin and eosin stain. × 420.

FIG. 8. Case 2. Putamen and globus pallidus, showing deposits of calcium in small capillaries and in the wall of a small vein. Hematoxylin and eosin stain. × 560.



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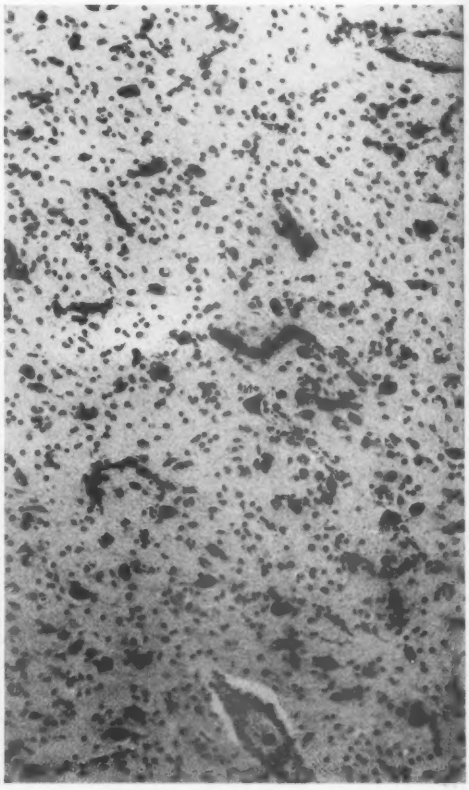

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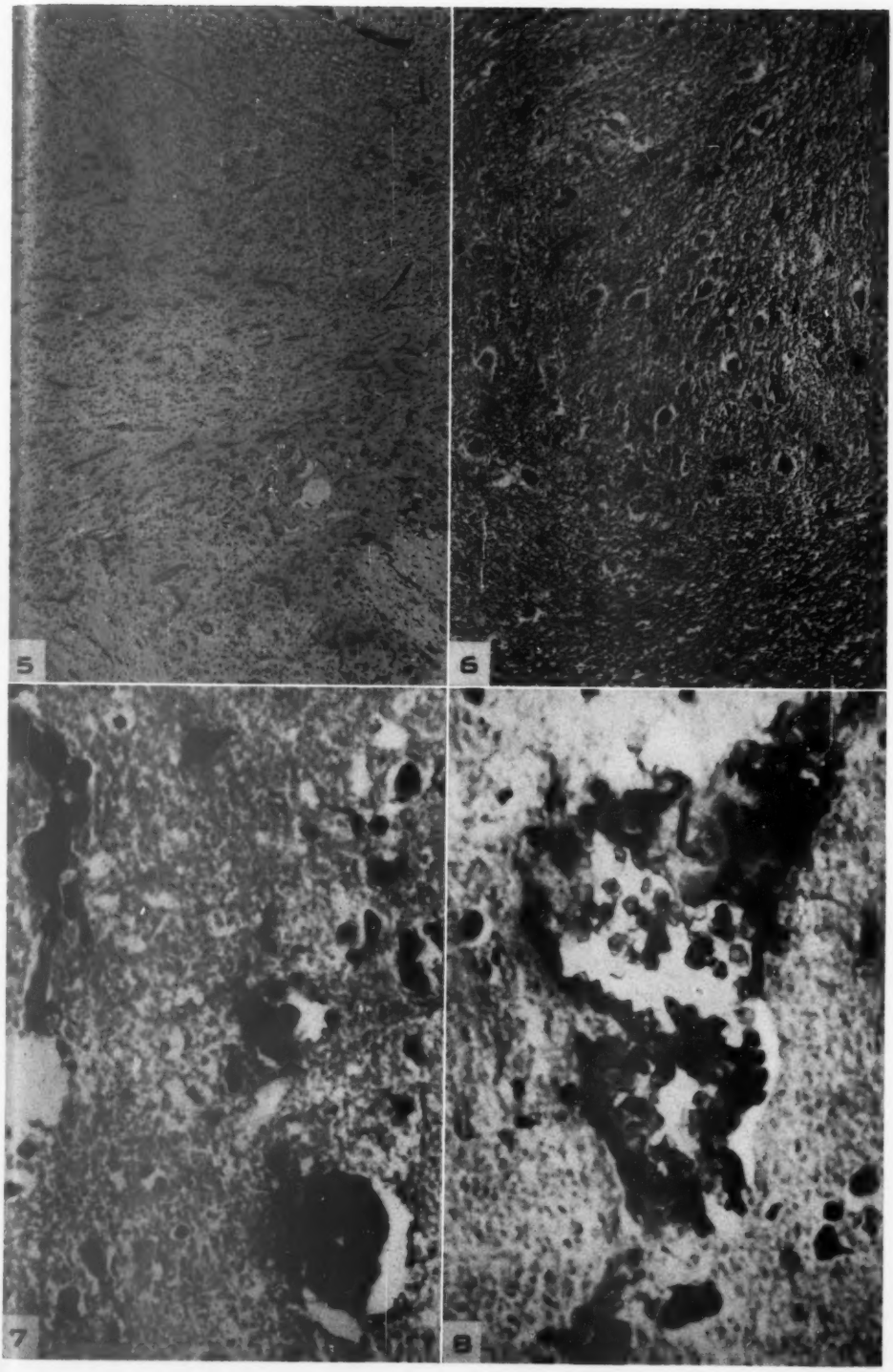
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HEREDITARY OCHRONOSIS

Pathologic Changes Observed in Two Necropsied Cases *

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Despite its comparative rarity, or perhaps because of it, and its spectacular nature as well, considerable interest has been evinced in ochronosis. The extent of the pertinent literature may be judged by the fact that over 100 articles were read in the preparation of this report. It is not feasible within a limited scope to attempt to survey the entire historical background, although references will be made to some of the more important contributions.

Since Virchow's [1] brief account in 1866 of the puzzling condition which he designated as ochronosis, some 200 cases, more or less, have been reported, although this does not begin to reflect the actual incidence of the condition because many instances undoubtedly go unrecorded. Many of these papers have been essentially clinical, dealing with the recognition and significance of alkaptonuria or the subsequent manifestations of the fully developed picture of the disease. Among the more informative of these papers, in the English literature at least, have been those of Osler,[2] describing the characteristic pigmentation of "cartilages, sclerotics, and skin"; of Smith,[3] stressing the diagnostic importance of the early ocular manifestations; of Pomeranz, Friedman, and Tunick,[4] emphasizing the specificity of the roentgenologic changes in the joints and the vertebral column; of Sutro and Anderson,[5] clarifying the nature of the peculiar arthritic alterations; and of Young,[6] pointing out the tendency to lithiasis of the genito-urinary tract, especially the prostate. By far the most valuable general summary of recent date is that of Galdston, Steele, and Dobriner,[7] dealing mainly with the metabolic aspects of the disorder. Many of the relevant accounts in standard texts seem quite inadequate, even after making allowance for the fact that they are necessarily brief.

Understanding of the pathologic changes in ochronosis has not kept pace with the slow, but steady progress in the delineation of the clinical picture. In relation to the literature as a whole, the number of necropsied cases has been rather limited and, of these, relatively few have been thoroughly documented and well illustrated. Perhaps the

* Received for publication, June 22, 1953.

most informative of the reports by American authors is that of Oppenheimer and Kline,[8] but even this does not provide altogether comprehensive coverage. It is the main purpose of this paper to describe the gamut of pathologic changes observed in fully developed ochronosis (as seen particularly in our two cases) and, at the same time, to bring the condition into sharper focus in the light of recent pertinent advances in genetics and biochemistry.

At the outset, some definition of essential terms seems indicated. There has been a tendency in the past to confuse alkaptonuria with melanuria[9] and phenoluria, or to lump them together as related expressions of ochronosis so-called, sometimes qualified as endogenous or exogenous, as the case may be. In this discussion alkaptonuria will be used to denote the spontaneous urinary excretion of homogentisic acid as one manifestation and usually the initial expression of an inbred disorder of phenylalanine and tyrosine intermediary metabolism, which may be appropriately designated as hereditary ochronosis. As such, alkaptonuria is no more a disease *per se* than is glycosuria in relation to diabetes mellitus. It is true that in medical journals and in standard texts one may find alkaptonuria and ochronosis discussed under separate headings, implying that there is not necessarily a positive association between them. It is difficult to conceive, however, how a patient with clinical ochronosis could fail to exhibit alkaptonuria at some time (provided the examination were properly performed), or, conversely, how a patient with genuine alkaptonuria could fail eventually, if he were followed sufficiently long, to exhibit discernible pigment deposition in the usual predilected sites. In this connection, also, one finds the oft-repeated, misleading statement that alkaptonuria (ochronosis) is a harmless disease. While the condition is compatible with longevity, it results rather regularly in more or less disabling arthritis and spondylitis, and not infrequently in significant cardiovalvular lesions, particularly aortic stenosis. One may mention also intensified arteriosclerosis, ochronotic nephrosis, and the formation of urinary calculi as additional deleterious effects of pigment deposition.

As indicated, we have chosen the designation of hereditary ochronosis. The prefix "hereditary" serves to emphasize the genetic defect constituting the basis for this disorder, and to distinguish it clearly from the comparable, though not entirely analogous condition of abnormal pigment deposition resulting from the prolonged administration of phenol-containing compounds.[10,11] Fortunately, such cases are now rarely observed, although at one time it was considered good prac-

tice to employ carbolic acid (in 3 per cent aqueous solution or as 10 per cent ointment) as well as picric acid (trinitrophenol) in antiseptic dressings for indolent wounds or ulcers. The resulting condition, sometimes referred to as "exogenous ochronosis," differs essentially from genuine hereditary ochronosis in that its progress may be arrested by cessation of the administration of phenols and in that the offending pigment found in the urine and deposited in excess in the tissues is chemically different from homogentisic acid (2,5-dihydroxyphenyl-acetic acid).

It has been recognized[12,13] for some time that alkaptonuria (hereditary ochronosis) results from an inborn error of metabolism, as Garrod[14] expressed it. Like other inbred disorders of metabolism, it is relatively infrequent, exhibits a familial pattern often associated with consanguinity, and manifests itself in early life. In mendelian terms, it is a comparatively rare recessive condition. According to Stern,[15] it occurs in approximately 1 of 1,000,000 persons, although it is carried in heterozygous state in 1 of 500, that is, by 2,000 times as many people as show the character. The incidence of ancestral consanguinity (mainly marriage of first cousins) among pedigrees collected in different countries has been estimated[15] to be as high as 30 per cent. In keeping with the congenital nature of alkaptonuria is the long recognized observation that affected infants manifest indelible staining of their diapers from birth.

Considerable insight has been gained also in regard to the *modus operandi* of the genetic defect in hereditary ochronosis, as expressed in biochemical terms. There is now experimental evidence to indicate that enzymes are under gene control and that single genes may control biochemical reactions through the mediation of specific enzymes.[16] Collaterally, inherited gene alterations or defects in genes may lead to loss of specific enzymes. It is believed that a certain gene in normal individuals controls the enzyme which makes possible the breakdown of the benzene ring of homogentisic acid, an intermediary metabolite of the amino acids phenylalanine and tyrosine, and its subsequent conversion to carbon dioxide and water, via aceto-acetic acid. In an alkaptonuric person this gene is apparently lacking, as is its dependent enzyme,[17] so that homogentisic acid cannot be metabolized and is excreted as such in the urine; while its excess is gradually deposited in certain tissues of the body as a peculiar pigment, with long-range effects which have already been indicated. It is interesting to note that hereditary ochronosis is only one of several recognized disorders of

phenylalanine and tyrosine metabolism stemming apparently from congenital enzymatic defects, others being phenylpyruvic oligophrenia, tyrosinosis, and probably albinism as well.

In the light of this hypothesis, one can more readily interpret the available data of metabolic studies relating to the problem of alkaptonuria. One can explain plausibly, for example, why urinary excretion of homogentisic acid in an alkaptonuric patient should be augmented by a diet rich in phenylalanine and/or tyrosine[18-20] and why, on the other hand, it cannot be altogether abolished, except perhaps temporarily, by the expedient of fasting.[21,22] It is significant, also, that appreciable reduction in the excretion of homogentisic acid in an alkaptonuric patient was noted by Leslie[23] after the administration of vitamin C (ascorbic acid), which seems to function as a co-enzyme. As is well known, excretion of homogentisic acid may be induced in white rats by a diet rich in phenylalanine,[24,25] as well as in scorbutic guinea-pigs.[26,27] The spilling over of intermediary metabolites (though not necessarily homogentisic acid) has likewise been observed under these circumstances in premature infants. In these and in other instances of experimentally induced excretion of homogentisic acid, whether in laboratory animals or human subjects, one is dealing not with an absence of essential enzyme, as is the case in hereditary ochronosis, but rather with temporary and reversible failure of the enzyme system in question through overloading of substrate and/or deprivation of co-enzyme.

Report of Cases

Case 1

*Clinical History.** The patient was a white male, 54 years of age, who was admitted to the hospital on December 9, 1946, because of findings indicative of coronary thrombosis with myocardial infarction. He was known to have had hypertension for at least 4 years. For the previous 2 years he had had dyspnea on exertion, some ankle edema, and episodes of loss of consciousness ("black-outs").

Since 1919 (age 27), he had been aware of progressive grayish blue discoloration of the skin, about the eyes, ears, and nose particularly, and of dark urine, which indelibly stained his underclothes. It was also recognized that his sweat-stained handkerchiefs showed peculiar yellow-gray discoloration. There was no history of the use of silver or of phenol-containing drugs to account for this. Since 1942, he had been increasingly troubled by arthritis, involving first the vertebral column and later the knees and the proximal interphalangeal finger joints, particularly.

Physical examination showed bluish pigmentation of the skin, which was especially marked over the ears, the bridge of the nose, and the cheeks, as well as the hands. Pigment deposits were also observed in the lateral portions of the conjunctivae. In addition, deformity and swelling were noted of the proximal interphalangeal joints,

* Some of the clinical aspects of this case have been reported by Coodley and Greco.[28]

some of the carpal joints, and both knees. Motion of the spine was distinctly limited.

Râles were audible over the mid-lung fields. The heart was enlarged, with the point of maximum impulse in the sixth interspace, 3.0 cm. to the left of the mid-clavicular line. The rhythm was regular. A loud, blowing systolic murmur was heard in the third left interspace, and this was transmitted throughout the chest. A harsh systolic murmur was also audible in the aortic area. The blood pressure was 110/90, and the pulse pressure never exceeded 25 mm. of Hg.

Urine specimens, which were dark on voiding, were observed to turn black on standing, and examination on a number of occasions established the presence of alkaptonuria. Albuminuria (2 plus) was noted also. Blood count revealed a moderate secondary anemia. Serologic tests for syphilis were negative.

On investigation of the patient's familial background, it was ascertained that his mother and father were first cousins, and that a brother had "diabetes" (possibly alkaptonuria).

The patient's hospital course is not particularly relevant. Suffice it to state that he failed to respond to treatment for his cardiac condition and died in congestive heart failure on the ninth hospital day.

Post-Mortem Examination

The body was that of a well developed, white male, measuring 60.5 inches in height and weighing approximately 145 lbs. The sclerae had a bluish hue and showed the deposition of stippled pigment lateral to the cornea. The skin appeared wrinkled and scaly and showed a peculiar, bluish discoloration, which was more pronounced in some areas. Specifically, there was conspicuous, confluent, butterfly dispersion of pigment over the bridge of the nose, spreading laterally over the malar prominences. Obvious stippled pigmentation also was observed over the supraciliary ridges, the hair-bearing portion of the upper lip, the angles of the jaws, and the pinnae of the ears. The cartilages of the ear appeared dusky and blue-purple. Over the shoulders, the abdomen, the trunk, the sacral region, and the legs, the pigmentation was mottled and less intense. On the other hand, there was marked discoloration of the dorsal surfaces of the hands, from the wrist to the finger tips, particularly over the metacarpal-phalangeal and the proximal interphalangeal joints. The hands appeared gnarled, and Heberden's nodes were present. The knee joints appeared swollen and a hard, egg-sized mass was palpable in the right popliteal space.

The few remaining teeth were brown and carious. The buccal mucosa was not pigmented.

The panniculus adiposus was thin and bright yellow. The musculature of the thorax appeared atrophic. There was distinct purplish pigmentation of the fascial planes and of the ligaments and tendons, wherever they were encountered. Readily discernible pigmentation also was noted in the walls of the arteries everywhere, both large and

small. Upon opening the thorax, all of the ribs, including their cartilages, the sternum, and the ensiform process were sharply outlined by virtue of purplish-black discoloration, although the bones themselves, apart from the periosteum and attached ligaments and tendons, were not discolored. There was a healed angulated fracture of the cartilage at the costochondral junction of the sixth rib anteriorly.

The serous cavities contained no free fluid. In the right pleural cavity there were fibrous adhesions between the diaphragm and the lower lobe.

The liver edge extended to the costal margin. The stomach was markedly distended. There was wide dilatation of the right inguinal ring, through which several loops of ileum had extended into a scrotal hernial sac.

Heart. The pericardium showed irregularly mottled pigmentation of its parietal and visceral layers. Over the left ventricle particularly, there were irregularly dispersed, depressed areas of gray-blue to purplish black pigmentation at sites of recent organizing myocardial infarction. The heart weighed 500 gm. It was globular and showed marked hypertrophy of its left ventricular wall, as well as concentric hypertrophy and dilatation of the right ventricle. The major coronary arteries were extensively calcified and discolored. Streaks of purplish pigmentation also were observed beneath the endocardium of the anterior wall of the right ventricle, and throughout the right ventricular aspect of the septum. The adventitia of the pulmonary artery was deeply pigmented. There was likewise mottled pigmentation of its intima. The commissural junctions of the pulmonary valve cusps and the annulus of the valve likewise had a purplish color. The left auricle was dilated; its wall was hypertrophied, and its endocardium, gray-white and thickened. The left ventricular wall presented an extensive recent subendocardial infarct, which appeared soft and yellow in places. Elsewhere, where the myocardium was undergoing fibrosis, it contained irregular deposits of pigment, as noted. In the aortic leaflet of the mitral valve, there was a large, calcified, flat, oval plaque, which was purplish black. The entire annulus of the mitral valve was irregularly calcified and pigmented. The calcification extended into the posterior leaflet and, in places, even to its line of closure. The aortic valve was stenotic and also showed extensive pigmentation and nodular calcification involving its entire annulus, its cusps, and, to a lesser degree, its line of closure. At its commissures, fusion had occurred. Also noted was aneurysmal dilatation of the left anterior sinus of Valsalva, which, at its upper limit, presented a zone of calcified intimal ridging.

The coronary ostia were widely patent. The coronary arteries, however, were severely sclerotic, pigmented, and irregularly calcified, and their lumina generally were irregularly narrowed. In the midportion of the anterior descending branch, in the terminal portion of the right main coronary artery, and in the circumflex arteries there were many points of almost complete occlusion by intimal plaques. There was evidence also of previous thrombosis, and several such sites appeared to have been recanalized.

The aorta was somewhat dilated, and its intima was heavily pigmented, especially at sites of ulcerated atheromatous plaques. The pigment deposits were particularly heavy in the saccularly dilated, ascending portion of the aorta. The adventitial coat also showed discoloration by pigment.

Respiratory Tract. There was bluish discoloration of the epiglottis. The thyroid and cricoid cartilages, which appeared calcified and ossified, showed blackening of the perichondrium. The tracheobronchial tree was filled with frothy white fluid. All tracheal and bronchial cartilage rings were deeply pigmented. Calcified lymph nodes were found in the hilar region, and in the lateral aspect of the left lower lobe there was a subpleural, calcified, anthracotic, primary tuberculous focus. Both lungs were relatively small. On sectioning, they showed chronic emphysema, pulmonary edema, and focal hemorrhages. There was no pigmentation of the pulmonary parenchyma.

Liver. The liver was firm and weighed only 1100 gm. Except for grooves on the right lobe, its surface was smooth and glistening. On sectioning, it presented a yellowish color and evidence of hyperemia. The gallbladder, biliary tree, and portal veins were not remarkable.

Spleen. The spleen weighed 140 gm. Its surface was purplish, smooth, and somewhat lobulated. On sectioning, the pulp was fibrotic, firm, and red, showing no apparent ochronotic pigmentation.

Kidneys. The kidneys together weighed 360 gm. The capsules stripped with relative ease, revealing finely granular, firm surfaces, presenting occasional small, purplish flecks. On sectioning, the parenchyma cut with increased resistance. The arcuate arteries were unusually prominent by virtue of intramural blackish pigment deposits and yellow intimal sclerotic plaques. The markings of cortex and medulla were indistinct. The pelves and ureters appeared grossly normal.

The urinary bladder was small and contracted, and its mucosa was bluish. Bulging into the prostatic urethra and almost occluding it, were a number of black, granular, mulberry-like concretions, which

apparently had been extruded from the prostate. Similar calculi were present also in the prostate, and the largest of these measured 3 by 2 by 1.5 cm. The prostatic tissue felt firm and indurated, as did also the seminal vesicles.

Adrenal Glands. The adrenal glands were not remarkable.

Pancreas. The pancreas was not unusual grossly, although it cut with increased resistance.

Gastro-Intestinal Tract. There was marked dilatation of the esophagus, and distinct hypertrophy of the muscle sphincter at the esophageal-cardiac junction. The stomach, as noted, was appreciably dilated, but its mucosa appeared normal. The duodenum, jejunum, ileum, appendix, and colon were not unusual.

Skeletal System. As noted, the ribs and costal cartilages were black (Fig. 7). The anterior ligaments of the vertebral column as well as the ventral aspect of the sacrum were likewise pigmented. The vertebral column appeared rigid and presented pronounced lordosis of the lumbar region as well as moderate scoliosis. In addition, there were marginal exostotic rings at the intervertebral disks, where pigment was concentrated (Fig. 10). At the lumbosacral joint, there was a particularly prominent exostosis. Section of the vertebral column revealed that the intervertebral disks, where they were still preserved, were impregnated by blackish purple pigment and were quite hard, though rather brittle. Also noted was a tendency to lateral protrusion of modified disk material and associated osteo-arthritis. At some levels (Fig. 11), the substance of the intervertebral disk had largely disappeared, leaving spaces bordered by ragged, brittle, purple-blue bone. At other levels, only discontinuous remnants of blackish disk tissue remained. At still others, where the entire disk had disappeared, the contiguous vertebral bodies had apparently fused (Fig. 13).

Examination of one of the swollen knee joints revealed extensive pigmentation of the articular capsule, as well as of the ligaments and tendons in its vicinity. There were prominent marginal exostoses. The synovial fluid had a faint gray hue. Innumerable fragments of black cartilage were found lying free within the joint. The cartilage of the ends of the articular bone appeared extensively eroded and inky black. Some of the defects in the articular cartilage extended to the subchondral bone. The synovium was everywhere thickened, villously transformed, and likewise deeply pigmented. It also presented numerous discrete and conglomerate, calcified, blackish chondral bodies embedded within and beneath its lining (Fig. 18). These were especially prominent within the lining of the posterior compartment of the knee

joint. Comparable changes were observed in the smaller joints examined, notably in the sternoclavicular joints and in the joint between the manubrium and body of the sternum (Fig. 6). The finger joints could not be dissected.

The symphysis pubis, too, was intensely pigmented, hard, brittle, and thickened, and presented nodular pigmented osteo-arthritic protrusions.

Brain. The dura and its sinuses exhibited purplish pigmentation. Also noted was discoloration of the periosteum of the inner table of the calvarium, but the bone itself was not altered. The brain weighed 1350 gm. Its external appearance was not unusual. The leptomeninges appeared normal, without apparent discoloration. The basilar and internal carotid arteries showed slight pigmentation and moderate arteriosclerosis, with appreciable narrowing. Sections of the cerebellum revealed symmetric cortical areas of encephalomalacia which were brownish blue. Serial sections of the cerebral hemispheres also presented occasional areas of encephalomalacia within the gray and white matter of the parietal and occipital lobes. These measured up to 1 cm. in width and were blackish purple.

Microscopic Examination

The significant findings have been incorporated in the anatomical diagnosis and for the sake of brevity will not be described in detail here, except for those relating to the manifestations of ochronosis.

The distribution of ochronotic pigment can be more readily recalled if an orderly grouping of the sites of predilection is attempted, as follows: (1) walls of blood vessels generally, and particularly the arteries of both large and small caliber, the arterioles, and capillaries (only scanty deposition in veins); (2) cartilages, generally: ears, nose, ribs, tracheobronchial tree, synovial joints, and synchondroses; (3) skeletal connective tissues: joint capsules, intervertebral disks, ligaments, tendons and their sheaths, fascia, periosteum, and perichondrium; (4) other, relatively collagenous or avascular connective tissues: in heart (pericardium, valve cusps and rings, organizing myocardial infarcts), scars and organizing infarcts elsewhere, conjunctiva, and dura mater; (5) excretory organs: sweat glands; urinary tract (renal tubules, bladder mucosa, prostate, and seminal vesicles); (6) endocrine glands: islets of pancreas, pineal gland, and pituitary body; (7) reticulo-endothelial system generally: lining endothelium, fixed tissue stellate reticulum cells and wandering histiocytes in skin, fibro-fatty tissue, liver, spleen, lymph nodes, bone marrow, adrenal sinusoids,

renal glomeruli and interstitium, thyroid gland, and submucosa of enteric and urinary tracts.

Whatever its site of deposition might be, the pigment appeared granular, rather than crystalline. Its color, as viewed in sections stained with hematoxylin and eosin, varied from yellow (ocher) through brown to blue-black, depending upon its concentration. While it might be intracellular, for the most part it impregnated the ground substance of the poorly vascular tissues, though in precisely what chemical form or combination is not as yet understood. When it did so, it eventually induced profound physical changes, as well as discoloration. The articular cartilages, for example, were rendered hard and brittle, and therefore were prone to splinter. In skeletal sites also, as is well known, calcium deposition tended to accompany pigment storage, although this was more apparent in roentgenograms of the affected structures than it was in hematoxylin and eosin sections, in which the purple cast of calcific deposits was obscured by the deeply pigmented background. In the walls of the larger arteries, the changes indicated were associated with accentuated arteriosclerosis, and in the heart valves with calcification, which has resulted not infrequently in aortic stenosis, as in this case. Apart from these and other specific significant effects which will be discussed presently, the deposition of ochronotic pigment *per se* provoked relatively little inflammatory reaction beyond the sluggish formation of occasional macrophages. The foreign body giant cells observed in relation to pigment were prone to be encountered within the lining tissue of joint capsules about fragments of fractured articular cartilage which had been ground into the synovium.

Anatomical Diagnoses. Hereditary ochronosis with alkaptonuria: pigment deposition in conjunctivae; sweat glands; blood vessels generally; cartilages of ears, nose, ribs, tracheobronchial tree and joints (ochronotic arthritis); intervertebral disks (ochronotic spondylitis with fusion of vertebral bodies); ligaments, tendons, tendon sheaths, fascia, periosteum, and joint capsules; heart (pericardium, myocardial infarcts, valve cusps and rings (aortic stenosis); convoluted tubules of kidneys (ochronotic nephrosis), bladder mucosa, prostate (pigment calculi) and seminal vesicles; pancreatic islets; dura, pineal gland, and pituitary body. Severe generalized arteriosclerosis; coronary thrombosis with old and recent myocardial infarcts (pigmented); cerebral arteriosclerosis with multiple old foci of encephalomalacia (pigmented). Scrotal hernia (right); papillary adenoma of renal cortex; lipogranulomatosis of spleen.

Case 2

For this instance of hereditary ochronosis in a male subject, 52 years of age, the record was found in old files. It was stated that the patient had passed a kidney stone 8 years previously, and that he had also had a salivary duct calculus. Enlargement of the heart had been noted on physical examination, as well as a loud, rasping, systolic murmur at the apex, a systolic murmur over the aorta, and a thrill over the entire precordium. The clinical impression as to his cardiac status was mitral insufficiency and aortic stenosis. At the time of his final admission, he was dyspneic, orthopneic, and had edema of his feet and ankles.

The other pertinent findings noted had reference to the presence of severe arthritis involving particularly the knees and hip joints, as well as the vertebral column, which showed lumbar lordosis and some kyphosis of the dorsal region. Motion of the spine was limited and painful. Roentgenograms (taken 5 years earlier) showed evidences of advanced osteo-arthritic changes in both hips and knee joints. Also noted were prominent marginal exostoses along the course of the vertebral column, associated with disruption and increased density of the intervertebral disks.

The protocol of the necropsy was of limited value, inasmuch as the prosector did not appreciate the significance of the gross changes as indicating ochronosis. Although nothing was said in regard to discoloration of the skin, the brief record did make mention of blackening of costal cartilages, the lining of the sternoclavicular joint, and the intervertebral disks. In addition, sections of the lung revealed dark brown pigmentation of the bronchial cartilages and their perichondrium.

The other pertinent findings mentioned have reference to the cardiovascular changes. The visceral pericardium showed hyaline thickening in places, and at these sites there was heavy pigment deposition, imparting a chocolate brown color to the gross specimen. The aorta showed pigment deposition microscopically, although no specific mention was made in the protocol of brown or black discoloration of other large or medium-sized vessels. Of particular interest was the finding of a blackened, scarred, and stenotic aortic valve, comparable to that observed in case 1 (Fig. 2). The myocardium in the vicinity of the aortic valve cusps was described as being black, also.

The remaining pathologic changes recorded were concerned with the finding of disseminated tuberculosis, which apparently constituted the primary cause of death.

Discussion

The major pathologic manifestations of hereditary ochronosis and their clinical reflection merit further consideration at this point.

Cutaneous Pigmentation

First, let us consider briefly what is commonly, though somewhat loosely referred to as pigmentation of the skin. Apart from the discovery of alkaptonuria and the more subtle ocular manifestations, this

is usually the earliest and most obvious expression of ochronosis. The age at which it becomes evident to the patient or his associates varies in individual cases, presumably with the severity and tempo of the disorder. In our first case, in which a meticulously detailed history was available, it was not until the age of 27 years that the subject became fully cognizant of gray-blue discoloration about the eyes, nose, and ears, which he ascribed to powder burns. It is interesting, in view of the intensity of this discoloration, that the epidermis and the cutis proper were not pigmented, as one might expect. The pigmentation noted externally actually reflected deposition of delicate, yellow-brown pigment granules within the endothelial cells of blood vessels in the skin and subcutis, within scattered macrophages of reticulo-endothelial derivation, and within the sweat glands and their basement membranes (Fig. 19). Minute quantities of pigment may actually find their way into the sweat, as evidenced by the clinical finding of staining by axillary sweat in occasional patients with ochronosis. Ochronotic pigment within the secretion of ceruminous glands has likewise been noted, although we did not observe this in our material. As indicated, the impression of cutaneous pigmentation also reflects, in large part, transmission through the integument of the coloration of heavy ochronotic deposits within the superficial cartilages, particularly of the ears and nose, and within the tendons and their sheaths where these lie close to the surface, as in the hands.

Skeletal Alterations

That the skeletal connective tissue structures bear the brunt of ochronotic pigment deposition is well known and has already been stressed. The bones themselves, apart from their articulations and their periosteal investment, are affected only to a limited extent. It is true that in proximity to involved joints and disks, and adjacent to the periosteum, one may find trabeculae in which the osteoblasts and their lacunae are stuffed with pigment and in which the matrix also shows brownish discoloration, but elsewhere the extent of pigment storage is insignificant. As for the alterations in the non-osseous skeletal tissues, those developing in synovial joints and in the intervertebral disks are of clinical importance, and their characteristic nature would seem to justify the specific designations of *ochronotic arthritis* and *ochronotic spondylitis* respectively, by way of emphasis.

In affected synovial joints, as indicated, the articular cartilage at the ends of the bones becomes hard and brittle, as well as pigmented, and tends to break away piecemeal in the course of articular function,

so that eventually the bone ends may be largely, if not entirely, denuded of cartilage (Fig. 14). When the involved joint is a comparatively large one, such as the knee, some of these pigmented cartilage fragments may constitute free joint bodies, as Sutro and Anderson[5] have demonstrated. Most of them, however, are ground into the synovium and the subsynovial connective tissue, producing the striking picture illustrated in Figure 18. Under these circumstances, it is not surprising that the synovium should show blackening and reactive villous synovitis (Fig. 15) or that with continued function (painful or limited as this may be), marginal exostoses, conspicuous subchondral sclerosis, and other changes of secondary osteo-arthritis of appreciable severity should ensue. Altogether, this sequence of events is reminiscent of what happens in a Charcot joint, although the mechanism leading to the development of innumerable intra-articular fracture fragments is quite different in ochronosis. While these arthritic changes develop slowly and usually become a clinical problem in middle age, pertinent cases have been recorded in which significant disability ensued long before the age of 40, and in one noteworthy instance,[29] symptoms of severe ochronotic arthritis involving finger joints and later other small joints, as well as the knees, began at the age of 13.

Equally important are the changes in the vertebral column, which develop concomitantly with the arthritic manifestations elsewhere, and lead eventually to a peculiar (ochronotic) ankylosing spondylitis. As noted, this has its essential basis in heavy impregnation of the intervertebral disks by ochronotic pigment. The distinctiveness of the roentgenologic changes reflecting such pigment deposition and its sequelae has been highlighted in a number of papers[4] stressing particularly the diagnostic significance of the striking radiopacity of the intervertebral disks (Fig. 12). The pigment is deposited throughout the disks, in the annulus fibrosus as well as the nucleus pulposus, and also in the contiguous longitudinal ligament, where it provokes the formation of prominent, more or less blackishly discolored, osteophytic rings (Fig. 10). Examination of specimens from far-advanced cases indicates further that the profoundly altered, impregnated disk tissue tends ultimately to be resorbed. At many levels, one may observe merely residual discontinuous remnants of it, mainly at the periphery, while the contiguous vertebral bodies manifest a tendency to fusion where the disk material is defective or absent (Figs. 11 and 13). The end result may be that of a substantially fused, "poker" spine, showing abnormal curvature. In the light of these changes, one can readily appreciate the

disability commonly associated with involvement of the vertebral column in ochronosis and the singularity of gait which Osler described almost 50 years ago.

Cardiovascular Manifestations

The effects of selective pigment deposition in the heart and blood vessels are as spectacular in their own way as those observed in the skeletal tissues. Moreover, they may be of clinical significance, as noted, in so far as they relate to accentuated arteriosclerosis of important vessels, *e.g.*, coronary and renal arteries, and to the development of valvular defects, especially aortic stenosis. These changes do not appear to be particularly well known, although in the initial case report of Virchow[1] mention was made of light gray discoloration of the intima of arteries generally, and of conspicuous blackening of the aorta at sites of atheromatous plaque formation.

From detailed study of the blood vessels in our material, the following observations may be noted. In the aorta, pulmonary arteries, and other large and medium-sized arteries, pigment granules are deposited rather early in the media, particularly in its outer portion, even in the absence of significant arteriosclerotic changes. Within the walls of arteries of both large and small caliber, one also observes a tendency to concentration of pigment along the medial aspect of the internal elastic lamella. Sites of intimal thickening associated with arteriosclerosis are not uniformly pigmented, although advanced lesions exhibiting necrotic plaques and fibrosis are deeply pigmented as a rule. Atheromatous plaques likewise may not become heavily impregnated with blackish ochronotic deposits until they are well established and ulcerated. As for the walls of arterioles generally, these are rather uniformly pigmented. Here, the pigment is likely to be interstitial. On the other hand, the pigment within the endothelial lining is mainly, though not entirely, intracellular. With reference to the veins, only occasionally does one observe delicate pigment granules within the media, and even these are so inconspicuous that one must search for them.

Where the pericardium shows appreciable fibrous thickening for whatever reason, ochronotic pigment may also be deposited in sufficient concentration to impart a brown or black coloration. Similar discoloration is likewise observed at sites of myocardial infarction undergoing fibrous repair (Figs. 3 and 4). In the heart valves, too, significant deposition of pigment occurs rather regularly. In the pulmonic valve in our first case, this was limited to purplish coloration of the annulus and of the commissural junctions. In the mitral valve,

the annulus was irregularly pigmented and calcified, while the anterior cusp was purplish black, as well as heavily calcified (Fig. 1). In the aortic valve (Fig. 2), intense pigmentation of the cusps and annulus, and of the sinuses of Valsalva, was also associated with appreciable calcification. Moreover, there was fusion of the commissures and obvious stenosis of the valve. It is apparently significant, and not mere coincidence, that aortic stenosis was found also in our second case. It may be added that in neither instance was there any indication of anomalous structure or previous rheumatic involvement as a basis for calcific aortic stenosis.

Renal Changes

Renal changes result in part from deposition of ochronotic pigment within renal arterial branches (the arcuate vessels in our case could be readily traced in the gross by virtue of their blackening), and its sequel of relatively severe arteriosclerosis. The presence of pigment-containing casts within renal tubules is also noteworthy, as is the observation of brownish pigment granules within the epithelial cells of the tubules, especially the convoluted tubules. This latter finding may be appropriately designated as ochronotic nephrosis (Fig. 17). Still another expression of urinary excretion of homogentisic acid, noted by Boyd,[30] is black discoloration of the renal pyramids.

Deposition of Pigment Within Endocrine Glands

Deposition of pigment within endocrine glands is a more subtle manifestation of ochronosis, which seems to have been largely overlooked. In the pancreas, particularly, on microscopic examination we observed rather conspicuous, selective pigmentation of the islets. These were relatively numerous and most of them showed the deposition of brown pigment granules to some degree (Fig. 20). Some of the pigment was undoubtedly contained within the islet cells, although much of it appeared to be within sinusoidal endothelial cells. Whether such pigment storage affects islet function significantly or whether it might lead eventually to diabetes is not known. To our knowledge, pancreatic islet function has not been studied in ochronotic patients by means of blood sugar estimations and glucose tolerance tests. From examination of the urine alone, it would take discernment to distinguish glucose from alkaptons, since both are reducing substances. In fact, patients with ochronosis are frequently thought to have diabetes mellitus when first observed, on the basis of routine urine examination revealing spurious "glycosuria."

Mention should also be made of the finding of brown pigment

granules within some of the cells of the pineal gland, as well as of both lobes of the pituitary body. The functional implications of such pigmentation, if any, remain a matter of conjecture.

Changes in the Central Nervous System

The dura, representing as it does a relatively avascular, thick, connective tissue membrane, is predilected, and in our case exhibited diffuse purplish pigmentation, as did also the walls of the dural sinuses. On the other hand, the leptomeninges were spared and the arteries comprising the circle of Willis were scarcely discolored, as compared with arteries of comparable caliber elsewhere. Of particular interest was the finding of pigmentation of organizing areas of encephalomalacia in the cerebral hemispheres and cerebellum (comparable to the pigmentation of organizing myocardial infarcts). As still another specific change, one may cite the observation by Boyd[30] of black discoloration of the choroid plexus of the lateral ventricles in a case of ochronosis.

SUMMARY

This paper deals with the characteristic pathologic changes in hereditary ochronosis, as indicated by the findings in two necropsied cases and collateral study of the pertinent literature. The prefix "hereditary" serves to emphasize the genetic (enzymatic) defect constituting the basis for this disorder of the intermediary metabolism of tyrosine, and to distinguish it clearly from the comparable, though not entirely analogous condition of abnormal pigment deposition resulting from the prolonged administration of phenol-containing compounds. Alkaptonuria is not a disease *per se,* but merely an early manifestation of hereditary ochronosis.

Far from being a harmless curiosity, as is often implied, hereditary ochronosis results eventually in more or less disabling arthritis and ankylosing spondylitis of a specific nature. The development of calcific aortic stenosis is another serious sequel, which was observed in both our cases. Still other deleterious effects are severe arteriosclerosis, ochronotic nephrosis, pigment deposition in the pancreatic islets, and the formation of pigment calculi in the urinary tract.

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LEGENDS FOR FIGURES

FIG. 1. Photograph (enlarged) of mitral valve in case 1, showing black pigmentation of the posterior portion of the aortic cusp, as well as of the posterior papillary muscle and the myocardium of the posterior wall of the left ventricle. The annulus also was discolored and calcified, although this is not apparent in the illustration.

FIG. 2. Photograph (enlarged) of aortic valve in case 1. There is extensive pigmentation and calcification of the entire annulus and the thickened cusps. The commissures are fused and the valve appeared stenotic before it was opened. Also evident is blackish pigmentation of the aortic cusp of the mitral valve, the myocardium adjacent to the aortic valve, the dilated sinuses of Valsalva, and the supravalvular portion of the aorta.

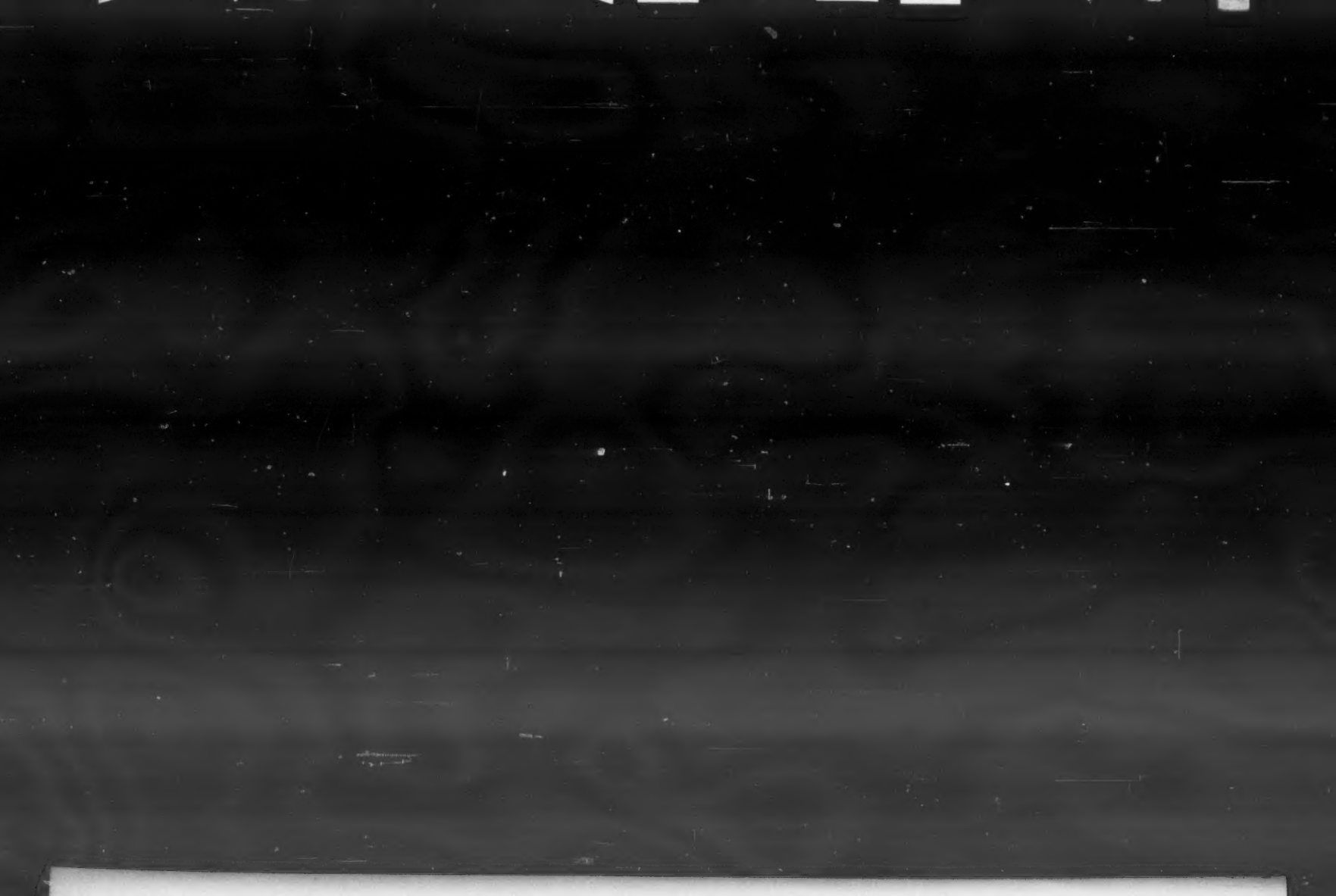

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FIG. 3. Photograph of slices of myocardium from the wall of the left ventricle, showing intense black pigmentation (at sites of myocardial infarction), as well as mottled discoloration of the papillary muscle.

FIG. 4. Photomicrograph of a field of the myocardium comparable to that illustrated in Figure 3. The coronary arterial branch is appreciably narrowed and its wall shows rather heavy pigment deposition. The dark portions at the top and at the lower margins represent areas of recent and organizing infarction in which ochronotic pigment has been deposited. × 50.

FIG. 5. Photomicrograph of a segment of the wall of a coronary artery, showing heavy deposition of pigment within an atheroma, as well as in the media. The black concentrations of pigment in the print appeared dark brown in the hematoxylin and eosin section. × 100.

FIG. 6. Roentgenogram of one half of the costal cage, including the sternum. The relative radiopacity of the costal cartilages may be noted as well as the pronounced osteosclerosis about the sternoclavicular joint and the joint between the manubrium and body of the sternum (reflecting reaction to advanced arthritic changes).

FIG. 7. Photograph (reduced) of the specimen illustrated in Figure 6, showing conspicuous blackish discoloration of the costal cartilages and their perichondrium, as well as of the periosteum of the ribs and the intercostal ligaments.

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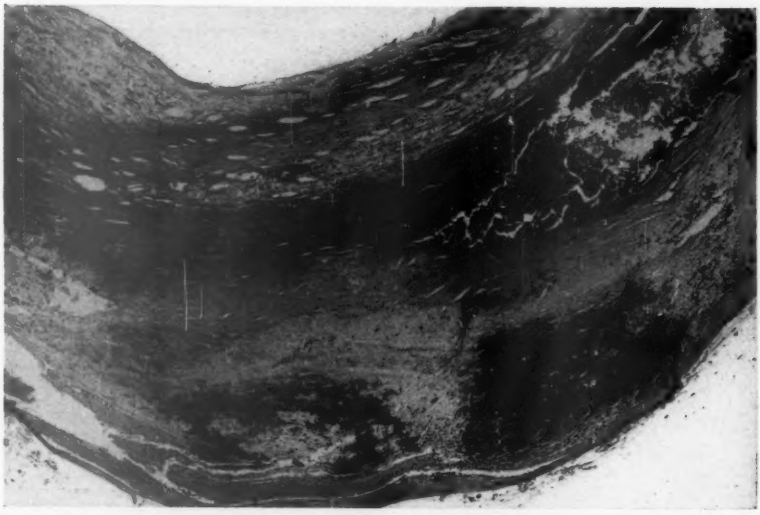

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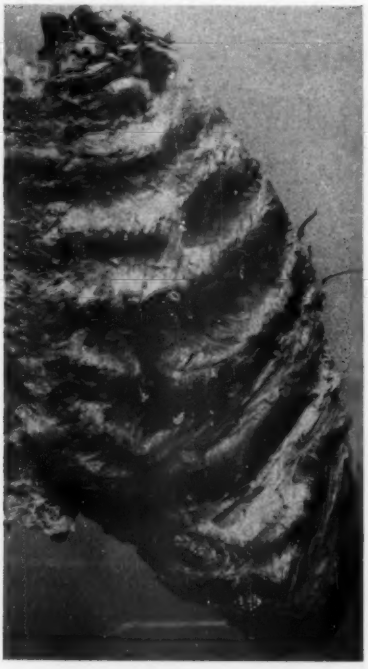

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FIG. 8. Photomicrograph of a section of the trachea, showing pigmentation of the cartilage matrix and the perichondrium. The latter contained dark brown pigment granules, as did also the walls of the small blood vessels. × 35.

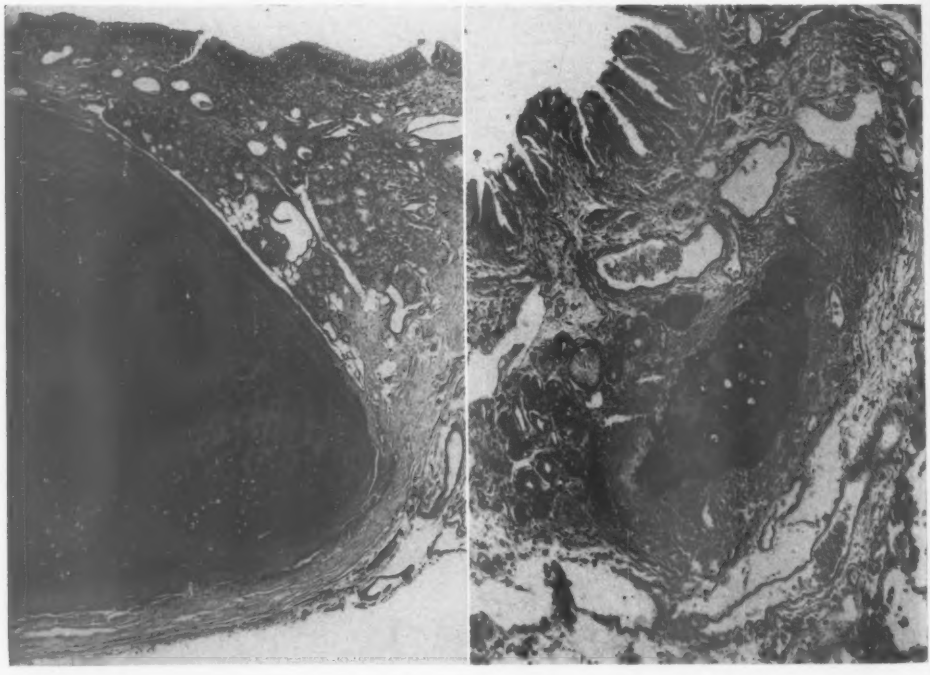
FIG. 9. Photomicrograph showing comparable changes in a bronchial cartilage and its perichondrium. Resorption of the cartilage apparently is associated with mild bronchiectasia. × 100.

FIG. 10. Photograph (reduced) of a portion of the vertebral column (lower dorsal and lumbar) as viewed externally, showing blackened osteophytic rings at the levels of the intervertebral disks.

FIG. 11. Photograph (reduced) of a section of the vertebral column, showing degeneration and pigmentation of the intervertebral disks. Some of the modified disks are still intact, while others show partial or virtually complete disappearance, with concomitant fusion of contiguous vertebral bodies. Of note also are the prominent marginal exostoses, and the prominent intervertebral space between D-12 and L-1.

FIG. 12. Roentgenogram of a segment of the lower lumbar vertebral column, showing conspicuous radiopacity of the intervertebral disks. Also evident are the arthritic changes in the lumbosacral joint at the distal end of the specimen.

FIG. 13. Photomicrograph of a section of the vertebral column, showing an involved intervertebral disk (see also Fig. 11). Most of the profoundly altered, impregnated disk tissue has disappeared at this level (presumably through resorption), and merely discontinuous remnants of it are seen. Where the disk is defective, the contiguous vertebral bodies have fused. × 4.



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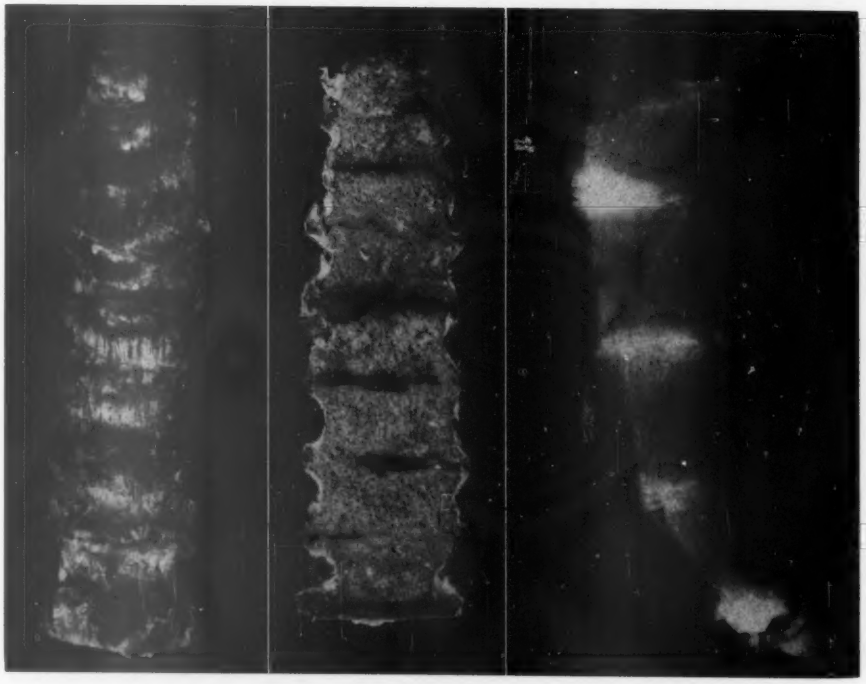

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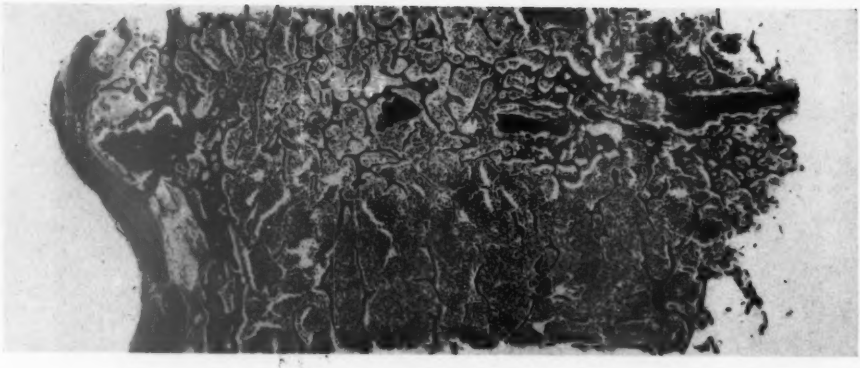

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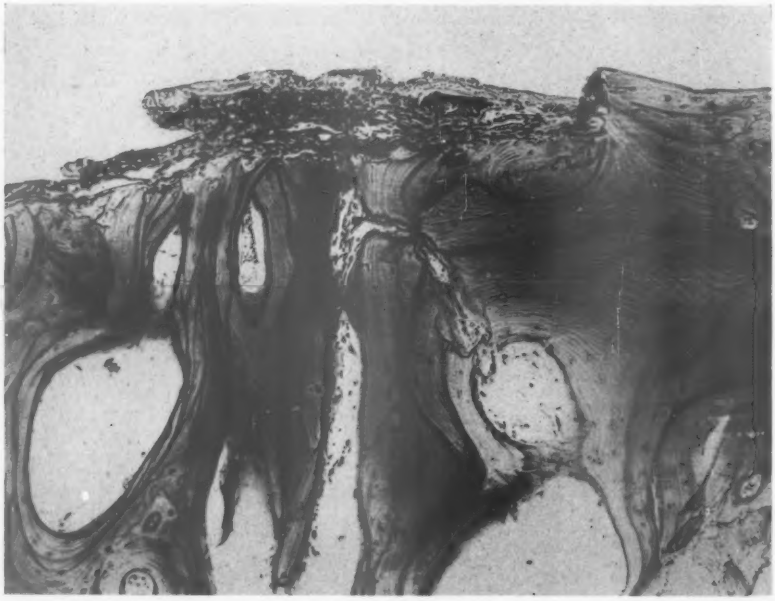
FIG. 14. Photomicrograph of an osteo-articular bone end, illustrating the peculiar arthritic changes observed in long-standing ochronosis. The modified cartilage has been completely denuded and replaced by intensely pigmented connective tissue. The pronounced sclerosis of the subchondral bone is an expression of secondary osteo-arthritis. × 25.

FIG. 15. Photomicrograph of synovial lining of an involved joint, showing chronic villous synovitis, as a reaction to fragments of pigmented articular cartilage within the joint and its synovium. The lining of a joint such as this showed dark brown to black discoloration grossly, depending upon the concentration of ochronotic pigment. × 60.

FIG. 16. Photomicrograph showing pigmentation of a tendon sheath, as well as reactive hyperplasia of its lining cells and foreign body giant cell reaction about particles of pigmented detritus. × 100.

FIG. 17. Photomicrograph of a representative section of a kidney, showing pigment granules within tubular epithelium (ochronotic nephrosis). Of note also are epithelial degeneration and desquamation, interstitial fibrosis, and pronounced arteriolar sclerosis. × 135.



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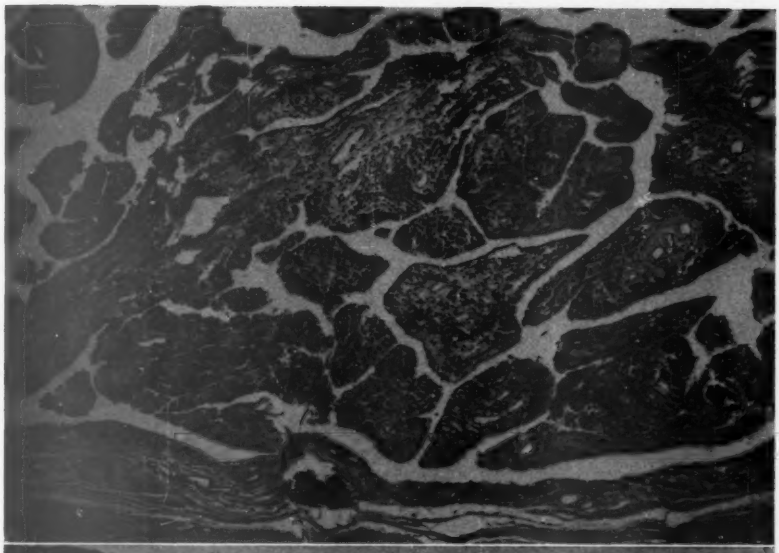

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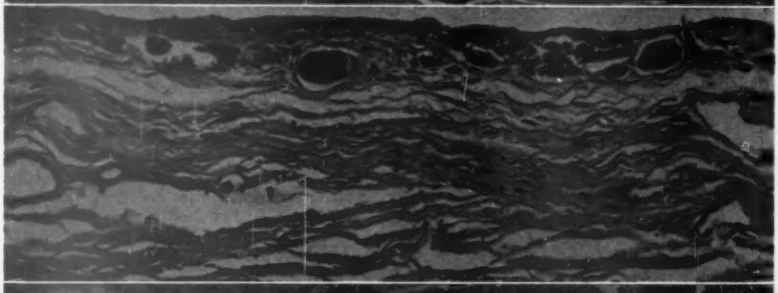

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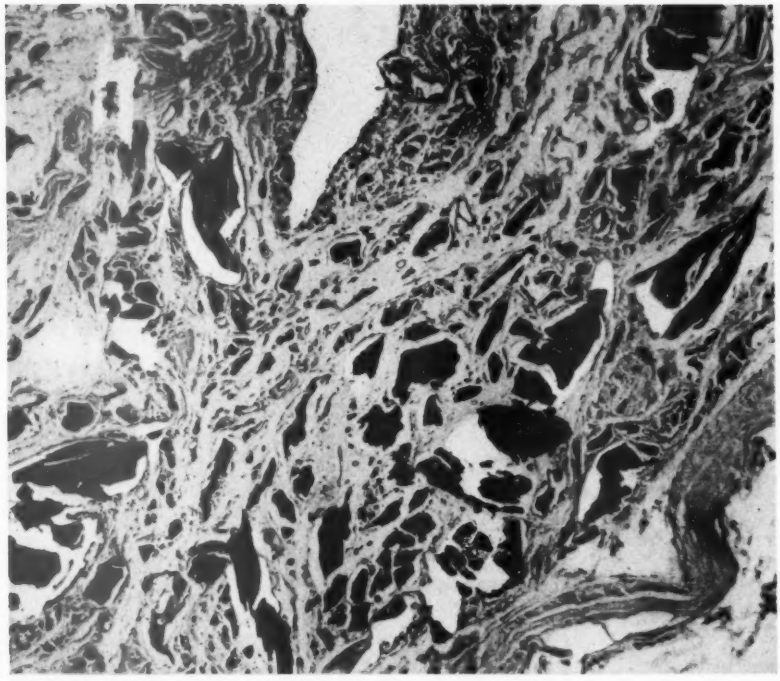
FIG. 18. Photomicrograph of a section of synovium of a knee joint, showing numerous fragments of broken-off, heavily pigmented articular cartilage ground into the synovial lining and the sublining tissues.

FIG. 19. Photomicrograph showing deposition of pigment granules within the sweat glands, both intracellularly and within their basement membranes. × 255.

FIG. 20. Photomicrograph of a representative field of the pancreas, showing selective deposition of (brown) pigment granules within the islet tissue. × 260.



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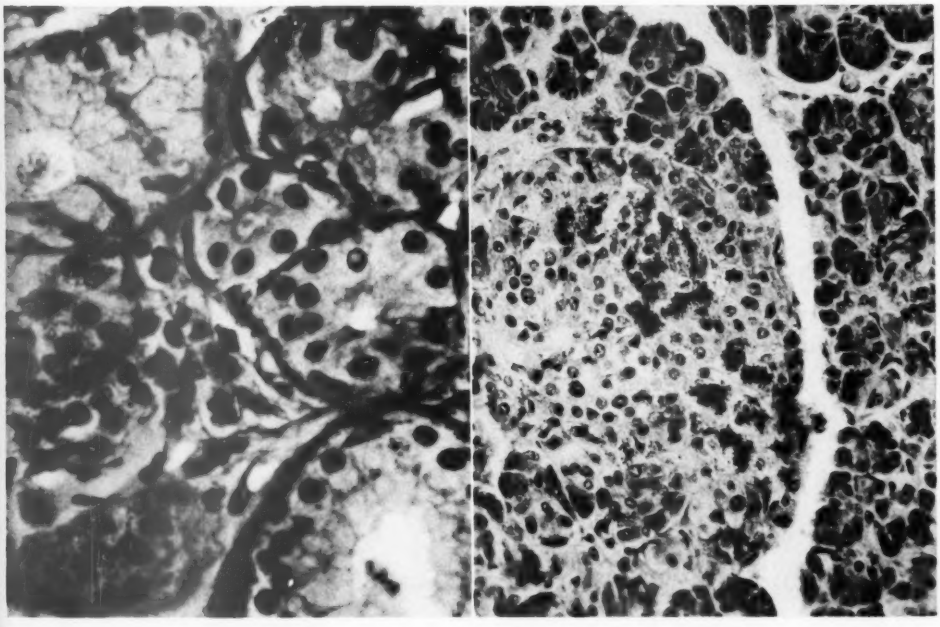

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THE NATURAL HISTORY OF EXPERIMENTAL GLOMERULONEPHRITIS PRODUCED BY FOREIGN PROTEIN *

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During the past 40 years numerous investigators have produced renal lesions in animals resembling in many respects the diffuse glomerulonephritis of man. There has not been, however, acceptance of the similarity of the human and experimental diseases, because of failure to clarify certain basic problems concerning the natural history of Bright's disease and of experimental glomerulonephritis. Our objective has been to follow the course of the renal lesions produced in rabbits by injection of foreign protein, and to determine how closely such lesions parallel those in man, in the hope that some light might be shed on the pathogenesis and course of the human disease.

The literature of the various types of experimental nephritis has recently been well reviewed by Kobernick[1] and Ehrich, Forman, and Seifer.[2] We have selected the method of repeated injection of massive doses of protein, which has been shown by Masugi and Sato,[3] Rich, Berthrong, and Bennett,[4] More and Waugh,[5] and Hawn and Janeway,[6] to produce diffuse renal lesions in rabbits. The incidence of such lesions in any group of rabbits may be variable, but can be increased by unilateral nephrectomy.[7] In addition to renal lesions, such animals show also a variable degree of arteritis and endocarditis. These lesions become evident approximately 1 week after administration of foreign protein. We are in agreement with the opinion expressed by Rich[8] that the cause of the lesions is hypersensitivity of the anaphylactic type. As the time required for the development of hypersensitivity is variable with administration of foreign protein, and the exact time and nature of the injury is unknown, two experiments were devised.

METHODS

Reversed Passive Anaphylaxis. Opie and Furth[9] produced reverse passive anaphylaxis in rabbits by injection of small doses of horse serum followed in 4 to 24 hours by injections of rabbit anti-horse serum. Accordingly, 6 rabbits weighing approximately 500 gm. were each given 1 cc. of horse serum intravenously, followed in 4 hours by the intravenous administration of 10 cc. of pooled rabbit anti-horse

* This work was supported by a grant from the National Research Council of Canada.
Received for publication, May 28, 1953.

serum. One rabbit died in 40 minutes. Two others went into shock but recovered. The remaining 3 showed no immediate effects. Immediate necropsy was done on the one animal, while the remainder were sacrificed by air embolism 3, 5, and 7 days later.

Repeated Injection of Foreign Protein. Fifty-one rabbits of approximately 2,000 gm. each were subjected to unilateral nephrectomy. They were then kept for 3 weeks, given rabbit chow and water *ad libitum*, and fresh vegetables twice weekly. At the end of this time each animal was given 20 cc. of sterile horse serum intravenously, followed in 10 days by the administration of 1 cc. of horse serum as a desensitizing dose. Eleven days after the initial injection, a further dose of 20 cc. of sterile horse serum was given. Groups of animals were then slaughtered by air embolism at intervals of 1, 2, 4, 8, 16, and 32 weeks after the second serum injection. The surgically removed kidney served as a control in each instance. For the sake of convenience, the renal lesions observed are dated from the time of the second injection, *i.e.*, 1, 2, 4, 8, 16, and 32 weeks. The size of each group is variable, as some of the animals died at irregular intervals, beginning with the second serum injection.

Histologic examination of heart, lungs, liver, spleen, adrenal glands, pancreas, gonads, mesentery, muscle, and kidneys was done routinely. Zenker's fixation was employed, and kidney sections were stained by Masson's trichrome stain, periodic acid-Schiff's reagent (McManus), phosphotungstic acid hematoxylin, cresyl violet, Laidlaw's reticulin stain, and hematoxylin and eosin.

No attempt was made to follow urinary and blood changes. This has been done by Waugh and More[10] and McLean *et al.*[11]

Results

Experiment 1. The animal dying 40 minutes following the injection of anti-horse serum showed congestion of pulmonary capillaries, patchy hemorrhages into alveoli, and some coagulated fluid in alveoli. The kidneys were markedly edematous, with distention of lymphatics and swelling of the walls of Bowman's spaces, which contained coagulated fluid. No hemorrhages were seen. Every cell in the sections appeared swollen and sometimes vacuolated: tubular epithelium, vascular endothelium, and muscle cells of arterial walls. Especially remarkable was the distention of the crescentic lymphatic space about Bowman's capsule. Of the remaining animals in this group, only 2 showed minimal renal changes. In one killed at 5 days, swelling of glomerular endothelium and epithelium was seen, and in one examined

at 7 days some proliferation of these same cells was noted. Similar changes were encountered in the first group of animals of experiment 2.

Experiment 2. In Table I is given the incidence of lesions at various intervals following the second serum injection. The only feature of possible significance is the high incidence of glomerular lesions during the first 2 weeks and the reduction in incidence of morphologic changes in animals kept 4 weeks or longer. This suggests resolution

TABLE I

Incidence of Arterial, Cardiac, and Renal Lesions Following the Second Serum Injection

Time in weeks	No. of animals	Arteritis and carditis	Nephritis
1	11	9	10
2	8	6	7
4	6	4	3
8	9	4	4
16	6	1	2
32	5	5	2

of some of the renal lesions within 2 to 4 weeks after the second serum injection.

The incidence of arteritis and carditis does not correspond to that of nephritis. Lesions of these two types do not bear any constant relationship. There was a similar reduction in arteritis and carditis after 2 weeks, with the exception of the last group in which all animals showed some evidence of either arteritis or carditis. No distinctive gross changes were encountered in any group.

HISTOLOGY OF THE RENAL LESIONS, 1 TO 32 WEEKS

One Week

The lesions found after 1 week were diffuse, affecting all glomeruli to a varying extent. Every component of the glomerulus was affected, including the afferent arteriole, the glomerular endothelium and basement membrane, the capsular epithelium, both visceral and parietal, and the parietal basement membrane. The connective tissue of the stalk also was involved, including the periarterial, or polkissen cells. It is our opinion that there is a delicate connective tissue mesangium in the rabbit glomerulus, dividing it into lobules. Secondary changes were seen in tubules. No vascular lesions other than those in the afferent arteriole were found.

The earliest alteration in the glomerulus appeared to be swelling of

endothelial, visceral epithelial, and connective tissue cells. Capillary basement membranes sometimes appeared broadened and blurred, but not disrupted. Hyaline droplets could be seen in epithelial cells covering the capillaries. The whole glomerulus appeared swollen, with reduction in caliber of the capillary lumina. Protein coagula could be seen in Bowman's space and in tubules. In proximal convoluted tubules, hyaline droplets similar to those in the glomerulus were encountered occasionally (Fig. 3).

A more severe reaction in the glomerulus was indicated by cellular proliferation, both endothelial and epithelial, with occlusion of capillary lumina by the endothelium. Connective tissue cells of the stalk as well as polkissen cells appeared increased, but the granule-containing juxtaglomerular cells did not. The basement membranes of the capillaries sometimes showed beading and thickening, but again their continuity could be followed. Bowman's space contained protein coagula and sometimes fibrin coagula, which could also be seen between capillaries of the glomerulus. Where the capillary lumina were not occluded by endothelial proliferation, the basement membranes often showed a thin adherent layer of protein and sometimes the lumina were occluded by protein coagula. The visceral epithelial cells showed swelling and slight proliferation only. Again, hyaline droplets could be seen in epithelial cells covering the glomerulus as well as in those lining Bowman's capsule (Fig. 4).

The most severe change resulted in disorganization of the glomerulus. The most striking feature of this was cellular proliferation: epithelial, endothelial, and mesangial, or of connective tissue. Both parietal and visceral layers of epithelium had proliferated, with obliteration of the capsular space. The basement membrane of the parietal layer usually showed fragmentation, splitting, and loss of continuity, so that sharp limitation of Bowman's capsule was lost. Instead, there appeared to be large, epithelium-like cells flowing out of the ruptured capsule into the adjacent periglomerular lymphatic. These latter cells, however, might have been proliferated endothelium of the lymphatic. The glomerular tuft showed compression due to crescent formation, or occlusion of capillary lumina by endothelial proliferation or by protein and fibrin coagula. Clots of fibrin often could be seen between capillary loops. The capillary basement membranes, like the parietal capsular basement membrane, showed beading, broadening, and fragmentation, becoming lost at times in proliferated cells which could not be differentiated as to epithelial or endothelial. Proliferation of cells about the afferent arteriole where it entered the

glomerular stalk also made delimitation of the glomerular capsule indistinct.

A few polymorphonuclear leukocytes could usually be seen in such glomeruli, while a few lymphocytes appeared to be intermingled with the epithelium-like cells around the glomerular capsule and the stalk of the glomerulus.

Convoluted tubules contained protein coagula, and, at times, red blood cells and occasional leukocytes. The lining epithelial cells showed hyaline droplets and swelling and vacuolation.

It should be pointed out that in each kidney in this group there was considerable variation in the severity of the lesions present, as changes ranging from the minimal ones described to the most marked disorganization of glomerular structure could be found in a single section. There was also considerable variation, in each instance, in the incidence of the most marked lesions.

Two Weeks

At the end of 2 weeks, not much alteration in the described lesions could be found. Cellular swelling had disappeared, and fibrin coagula in the capsular space appeared to be fragmenting and breaking up, as they were encountered less frequently. Cellular proliferation was still present, the connective tissue cells being more readily recognizable because of their deeper staining cytoplasm. The broadening and thickening of capillary basement membranes was more readily seen (Figs. 6 and 7). The capsular crescents showed beginning collagen formation. The glomerulus was still leaking protein, as pale-staining coagula were present in the capsular space and in convoluted tubules. Hyaline droplets were still present in glomerular epithelium, both visceral and parietal, and in the epithelium of convoluted tubules. Marked vacuolation of tubule lining cells could be seen. In collecting tubules, epithelial casts were found.

It should be pointed out that in this group, too, the extent of glomerular involvement was variable in that sometimes only one lobule or part of the glomerulus would show well marked changes, whereas other lobules would exhibit complete disorganization of the whole tuft. By partial involvement of a glomerulus is meant cellular proliferation in only one or two capillary loops, with or without overlying epithelial proliferation and adhesion to the parietal capsule.

Four Weeks

In the group of animals maintained for 4 weeks it was apparent

that resolution of some of the lesions had occurred, in that not only was the incidence of glomerular changes reduced, but the number of glomeruli involved appeared reduced in most of the kidneys examined. Histologic evidence of resolution included the finding of greatly distended capillary loops in many glomeruli. These appeared several times the normal diameter. The basement membranes of such distended loops appeared slightly thickened, and the mesangial or connective tissue cells appeared denser and more prominent. Otherwise these glomeruli appeared normal (Fig. 8).

Morphologic alterations still present appeared irreversible. The proliferative changes included the formation of adhesions between capillary loops and Bowman's capsule by epithelial cells, with obliteration of many capillaries leaving only a few patent lumina surrounded by capsular epithelium and collagen strands containing lacunae, all that remained of Bowman's space. More advanced lesions were represented by fusion of a hyalinized tuft containing a few nuclei with surrounding concentric collagenous tissue, the remains of the epithelial crescent. When these latter changes did not progress to complete obliteration of the glomerulus, they were associated with marked reduction in capillary filtering surface. The resemblance of these lesions to those of proliferative, or extracapillary glomerulonephritis (Ellis, type I[12]) was striking (Fig. 9).

The proliferated polkissen cells also appeared to have produced collagen, with fibrosis about the glomerular stalk. Similarly, the demarcation of Bowman's capsule from surrounding interstitial tissue was lost, suggesting that the increased cells, previously described as outside of Bowman's capsule, had also laid down collagen.

Reticulin stains revealed that the connective tissue or mesangial cells, which appeared more prominent, were surrounded by broader and thicker silver-staining fibrils than in the normal rabbit glomerulus. There was, however, only slight to moderate increase in such fibrils within the glomerulus. It was evident, too, that the proliferated epithelium of the glomerular tuft had not produced collagen, while the proliferated epithelial cells lining Bowman's capsule had.

In addition to these proliferative changes, alterations best labelled degenerative were seen. In some of the areas of cellular proliferation, both within lobules of the glomerulus and also in the cellular adhesions continuous with Bowman's capsule, the cytoplasmic outlines of the cells were indistinct and obscured by the presence of rounded clusters of hyaline droplets of varying size. Such droplets were variable in

staining qualities, being either red-orange or pale green with Masson's trichrome, and dark or light pink with McManus' stain (Fig. 10).

Varying degrees of tubular atrophy and degeneration were found, secondary to the glomerular changes.

Eight Weeks

At 8 weeks, progression of the lesions described at 4 weeks was noted. There were scattered atrophied and hyalinized glomeruli, with periglomerular fibrosis.

In one animal, which died spontaneously at 8 weeks, there were nodular hyaline masses in almost every glomerulus examined. These were reminiscent of the Kimmelstiel-Wilson lesion in diabetes mellitus. The hyaline material stained pale pink with McManus' stain, and pale green with Masson's trichrome stain, and light brown with phosphotungstic acid hematoxylin. It was not metachromatic with cresyl violet. Sometimes a patent capillary could be seen coursing around the periphery of these hyaline masses. At other times the hyaline material flowed outwards from the main mass to produce thickening of capillary walls. Within the main masses of hyaline material, cell nuclei could be seen, although these appeared hyperchromatic and sparse. The afferent arterioles occasionally showed similar hyaline thickening of their walls. Evidence of altered permeability of the hyalinized glomerulus was given by the presence of fibrin and plasma coagula in Bowman's space and in tubules, and by the presence of glassy hyaline droplets in both glomerular and tubular epithelium. There also appeared to be considerable atrophy of tubules associated with the markedly hyalinized glomeruli. The spleen in this animal contained a small amount of amyloid.

Sixteen to Thirty-Two Weeks

The 11 animals in the two groups maintained for 16 to 32 weeks are considered together as the lesions encountered were similar throughout.

Hyalinized shrunken glomeruli were commonly seen, surrounded by fibrous tissue and atrophic tubules. Hyaline change, similar to that described in the one animal at the end of 8 weeks, was found in several animals after 16 and 32 weeks respectively. It was not as extensive, however, in any one case and consisted of circular or oval masses of hyaline material usually confined to only part of a glomerulus. A more striking morphologic alteration was diffuse hyaline thickening

of capillary basement membranes in the glomerulus. This resembled very closely the type II nephritis of Ellis.[12] The thickened glomerular walls gave the same tinctorial reactions as the hyaline nodular masses. Brightly eosinophilic hyaline droplets, both intracellular and extracellular, were found in the glomeruli, and protein coagula were seen in Bowman's space and in convoluted tubules. It could not be determined whether the hyaline droplets occurred in endothelial or epithelial cells, or in both.

Frequently adhesions of glomerular tufts to Bowman's capsule were seen, usually in association with hyalinization of a lobule or part of a glomerulus. Similarly, some periglomerular fibrosis was present, usually about small shrunken glomeruli. Scattered areas of scarring containing atrophic tubules and sparse infiltrations with lymphocytes were encountered. No changes were seen in arterioles or in larger arteries.

Discussion

It is evident from experiment I that the injury to the kidney caused by a reversed passive anaphylaxis was widespread. Not only were glomerular capillaries injured, but also their covering epithelium, the parietal epithelium of Bowman's capsule, Bowman's capsule itself, the lymphatics, and the smaller blood vessels throughout the kidney. Further vascular injury was found in the lung. Experiment I is of value in that it demonstrated the extent of the injury in the kidney. It is our opinion that the injury resulting from a reversed passive anaphylaxis is similar to that resulting from repeated injection of antigen, as the histologic changes in the glomeruli were the same 1 week after the injury in both experiments.

In experiment II, beginning at 1 week and extending through 32 weeks, the changes described in the kidney all represent reaction to injury, that is, reaction to an injury which occurred some time during the week to 10 days after the first serum injection,[6] and which was enhanced by the second serum injection. It is apparent, in our opinion, that the reaction varies according to the degree of the injury. For example, the most minor degree of reaction was swelling of the components of the glomerular tuft alone, the endothelium, epithelium, connective tissue cells of the stalk, and the capillary basement membranes. The injury was somewhat more severe when cellular proliferation, endothelial and epithelial, was evident in the glomerulus, and possibly in Bowman's capsule. If the continuity of the basement membranes was maintained, it is our opinion that resolution of the lesion could occur. That is, cellular proliferation both of capillary endothe-

lium and covering epithelium would regress and disappear, with restoration of continuity of the capillary lumen. Similarly, we believe that the protein coagula obstructing capillary lumina could be resolved. Evidence for the above statements is indirect only, consisting of reduction in incidence of glomerular lesions 4 weeks after the injury, the finding for the first time of dilated glomerular capillaries with slightly thickened basement membranes and more prominent mesangial cells 4 weeks after the injury, and the fact that the progressive proliferative lesions were all characterized by loss of continuity of capillary basement membranes.

There is neither direct nor indirect evidence with regard to the resolution of lesions of Bowman's capsule. Proliferation of parietal epithelium was seen principally in association with rupture of the underlying basement membrane and with increase in pericapsular cells. This latter lesion progressed to periglomerular fibrosis, the formation of adhesions, and in extreme cases to apparent compression and obliteration of the glomerulus, as the proliferated capsular epithelium produced collagen. It is possible that proliferation of capsular epithelium in the absence of rupture of the basement membrane could regress with resolution and restoration of the capsule to normal, but we lack good evidence in support of this.

Although the most striking feature of all the early lesions observed was cellular proliferation, the most significant changes were related, in our opinion, to the basement membranes of the glomerular capillaries. Whether or not resolution occurred, appeared to depend upon the integrity of this membrane. In animals observed 4 weeks after injury, at which time it was apparent that all the lesions observed were progressive, two distinct types of reaction were noted. The first and most striking feature at this time was cellular proliferation with adhesions of capillaries to capsule, organization of crescents, and the thickening of the glomerular stalk and lobules by increased cells. Some basement membranes appeared highly refractile and thickened. However, in a single glomerulus, together with cellular proliferation one could also see the changes we have labelled degenerative, that is, the accumulation of amorphous hyaline material in the form of droplets within the cytoplasm of proliferated cells of the glomerulus. Capsular, or parietal epithelial cells did not show similar accumulations of hyalin. As previously stated, this material stained pale green with Masson's trichrome and pale pink with McManus' periodic acid-Schiff's stains. Whether or not it is the same material as the brilliantly eosinophilic droplets found in the earliest stages of injury and later

within endothelium and epithelium is questionable. These latter droplets were commonly found, at all stages of injury, within the glomerular cells as well as within proximal convoluted tubular epithelium, and were interpreted as protein which had passed through the glomerulus, some being resorbed en route, some being resorbed in the tubule, and some forming protein casts. In a few glomeruli could be seen hyaline droplets showing a gradual change in staining reaction from a deep purple to pale pink, with the periodic acid-Schiff's stain, and from bright red to pale green with Masson's trichrome.

In the animals sacrificed later than 4 weeks after injury, the most striking feature was the accumulation of this hyaline, amyloid-like material in the glomerulus. It was apparent that the progressive lesions described at an earlier stage had resulted in either complete fibrosis of the glomerulus and atrophy of the associated tubule, or when only a part of the glomerulus had been irreversibly injured, in hyalinization of the affected part. The evidence is suggestive, from observations of similar hyaline droplets in proliferated cell masses at 4 weeks after injury, that there was a progressive transformation of the proliferated glomerular cells (both epithelial and endothelial) into hyalin. Similar hyaline material produced diffuse thickening of capillary basement membranes, too, in many of the affected glomeruli, although how this change was mediated was not clear. Change in basement membranes was observed as part of the earliest lesion at 1 week, but at that time it took the form of broadening and blurring, or fragmentation, without any alteration in staining qualities. At the end of 2 and 4 weeks, thickened basement membranes appeared rather glassy and refractile, but without tinctorial alteration. The late change, with marked hyaline thickening and alteration in staining characteristics, does not resemble the earlier change at all.

From the foregoing, then, it is evident that in experimental glomerulonephritis of this type there can be a transformation of the lesions from a proliferative type to a degenerative type characterized by hyalinization not only of proliferated cells, but also of capillary basement membranes.

It is our opinion that human glomerulonephritis and the experimental type, as herein described, have much in common. The nature of the reaction to injury in the glomerulus and its capsule is fundamentally the same in both, although in the human disease endothelial proliferation is more prominent than visceral epithelial proliferation. Capsular reaction is the same in both, although one does not see pericapsular cellular accumulations in the human type. Resolution of the

human lesions does occur, and progression of the lesions results in fibrosis and hyalinization, although the nodular distribution of hyalin is not typical of diffuse glomerulonephritis. Ellis[12] described two types of glomerulonephritis: proliferative (type I) corresponding to the earlier lesions herein described, which ended in fibrosis; and membranous (type II) which resembles closely the very late stage lesions of the experimental type. In this investigation it is evident that in the experimental nephritis, transformation of proliferative to membranous lesions did occur. This appeared to be a direct change through the accumulation of hyaline material in small droplets, with gradual atrophy of surrounding cells. The fact that hyalinization was of late occurrence, from 8 to 32 weeks after the second serum injection, implies either a continuing injury with an altered response, or the induction of a progressive change in the glomerulus. The droplet form of this material, together with the late, very marked thickening of capillary basement membranes, is suggestive of an infiltration of altered protein which has many of the characteristics of amyloid. It may, on the other hand, be a product of the cells of the glomerulus, as suggested by Teilum *et al.*[13]

Summary

Reversed passive anaphylaxis, induced in the rabbit with horse serum and anti-horse serum, resulted in one animal in acute injury to the renal arterioles and small arteries, lymphatics, glomeruli, and tubules, and in two others in proliferative glomerular lesions seen 1 week after injury.

Repeated injection of horse serum in unilateral nephrectomized rabbits resulted in diffuse glomerulonephritis, characterized in its earlier stages by a variable degree of swelling and proliferation of glomerular endothelium, of covering epithelium, of the mesangium, and of parietal epithelium, and by swelling, fragmentation, and rupture of basement membranes of glomerular capillaries and Bowman's capsule. There was also marked accumulation of cells about Bowman's capsule, and about the afferent arteriole.

Resolution occurred in many cases between 2 and 4 weeks after injury. Indirect evidence was presented to show that resolution depended upon the integrity and continuity of capillary basement membranes, and possibly upon continuity of the parietal capsular basement membrane as well.

With disruption of basement membranes, proliferated epithelium lining Bowman's capsule laid down collagen, with ultimate periglomer-

ular fibrosis and usually obliteration of the glomerulus. With disruption of glomerular capillary basement membranes and accompanying proliferation of epithelium and endothelium, progression of the lesion was characterized by ultimate nodular hyalinization. These latter changes sometimes involved only part of the glomerulus, although 32 weeks after the last administration of foreign serum not only was nodular hyalinization of glomeruli noted, but also diffuse hyaline thickening of glomerular basement membranes, resembling a membranous nephritis (Ellis, type II).[12]

The similarity of the course of the lesions of experimental glomerulonephritis and human glomerulonephritis is discussed.

We are indebted to Dr. Neville Crowson for valuable assistance in conducting the first experiment, and to Professor Robert H. More for his advice and criticism in the preparation of this paper.

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[*Illustrations follow*]

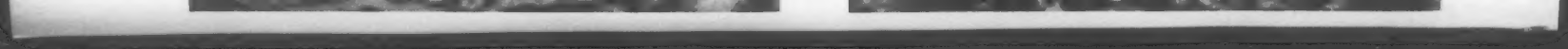
LEGENDS FOR FIGURES

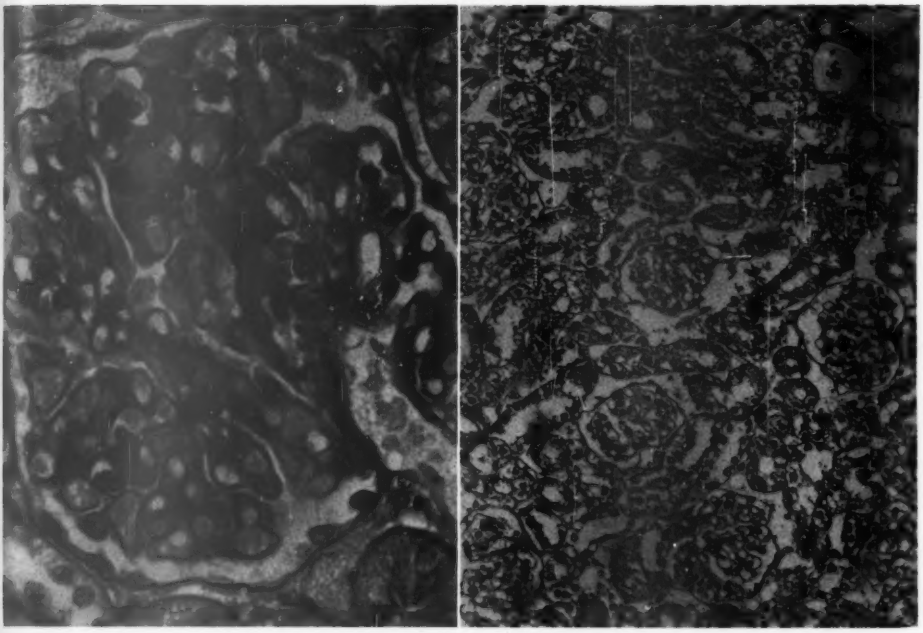
FIG. 1. Normal rabbit glomerulus. Periodic acid-Schiff's stain. × 538.

FIG. 2. Experiment 1. Reversed passive anaphylaxis. Death 40 minutes after the injection of antiserum. Swelling and vacuolation of cells, distention of lymphatics, especially the periglomerular semicircular lymphatic. × 96.

FIG. 3. Experiment 2. One week. Minimal glomerular reaction with swelling of epithelial and endothelial cells. Hyaline droplets in cells. Blurring and broadening of basement membranes. × 538.

FIG. 4. Experiment 2. One week. More marked reaction with proliferation of cells, both endothelial and epithelial in the glomerulus, and increase in cells about the capsule and stalk. × 256.





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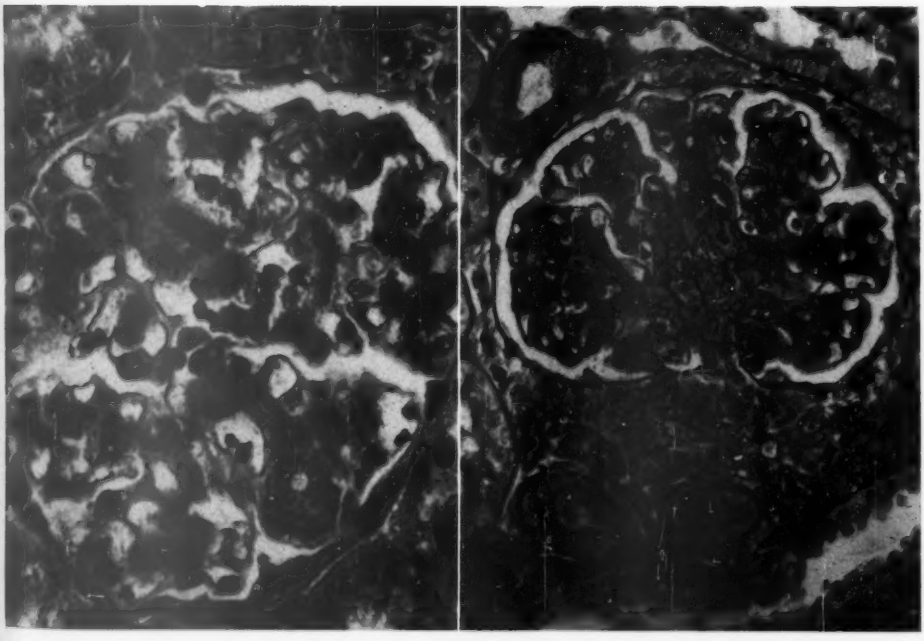

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FIG. 5. Experiment 2. One week. Most marked reaction with proliferation of epithelium, both visceral and parietal, capillary endothelium, and periarterial and pericapsular cells. Disruption of basement membranes of the capillaries and of Bowman's capsule. × 538.

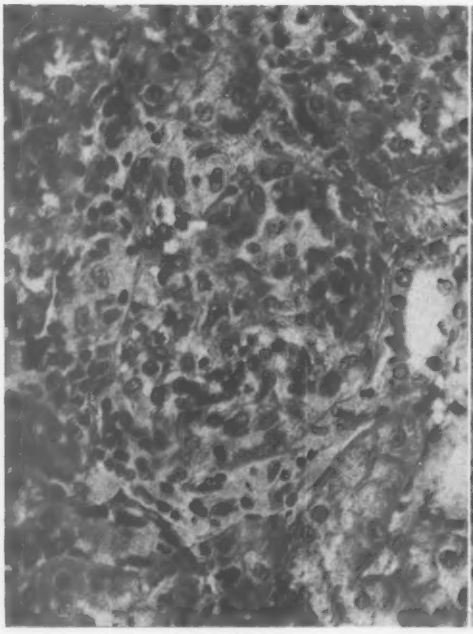
FIG. 6. Experiment 2. Two weeks. Proliferation of endothelium and visceral epithelium with some disruption of capillary basement membranes and obstruction of capillary lumina. × 404.

FIG. 7. Experiment 2. Two weeks. More marked cellular proliferation, including proliferation of parietal epithelium and pericapsular cells. × 192.

FIG. 8. Experiment 2. Four weeks. Clearing of glomerular capillaries, leaving irregular dilated loops, thickened basement membranes, and a few epithelial adhesions. × 404.

FIG. 9. Experiment 2. Four weeks. Late proliferative changes with capsular adhesions and organized crescent. × 404.

FIG. 10. Experiment 2. Four weeks. Late proliferative and degenerative changes with capsular adhesions and beginning hyalinization of proliferated cell masses. Hyaline droplets in parietal epithelium. × 404.



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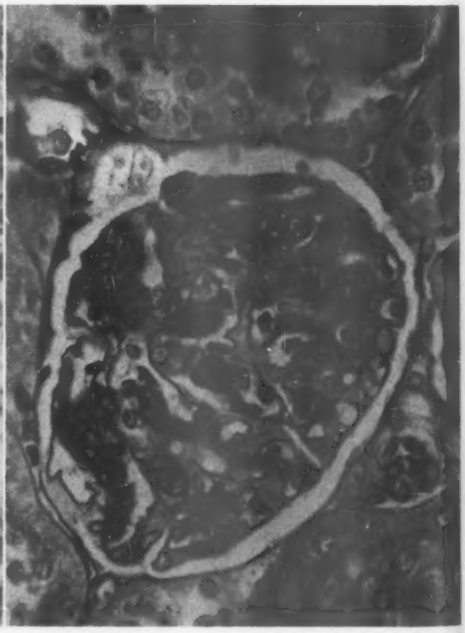

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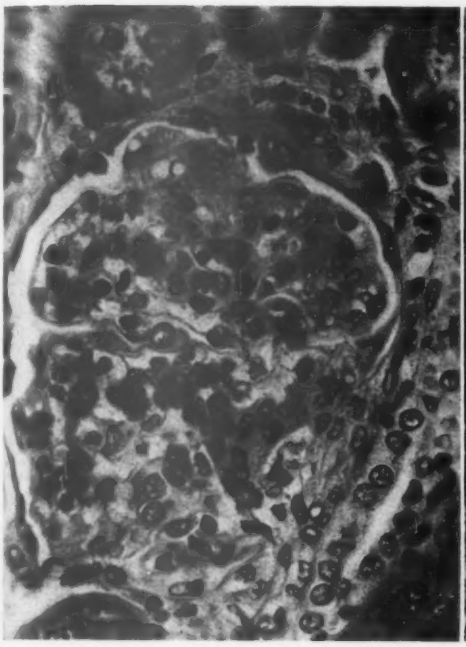

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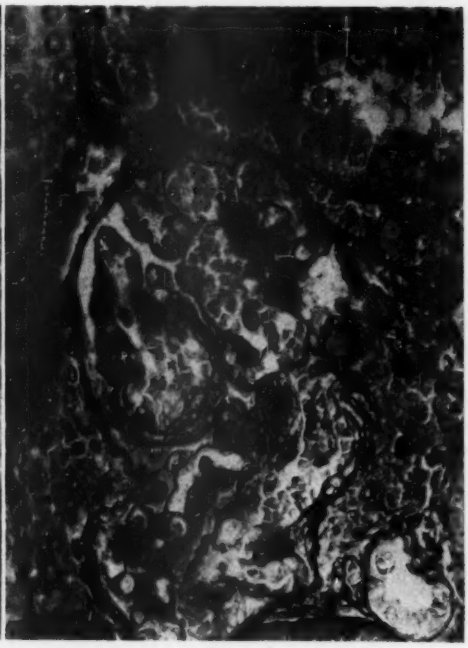

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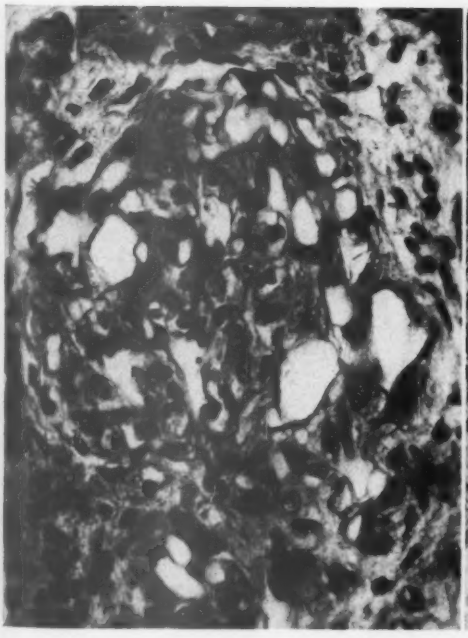

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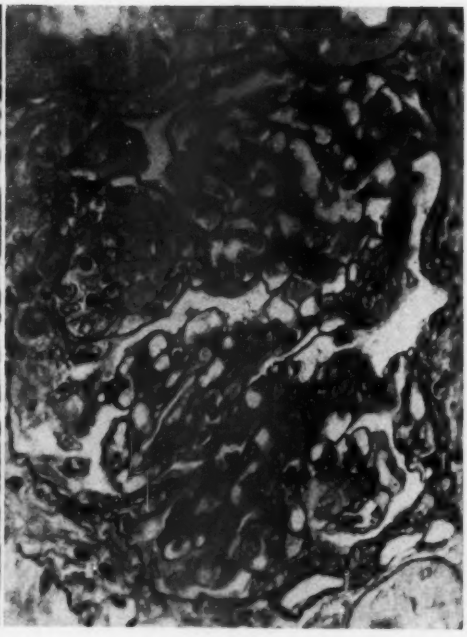

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FIG. 11. Experiment 2. Eight weeks. Old organized crescent with shrunken glomerulus. × 404.

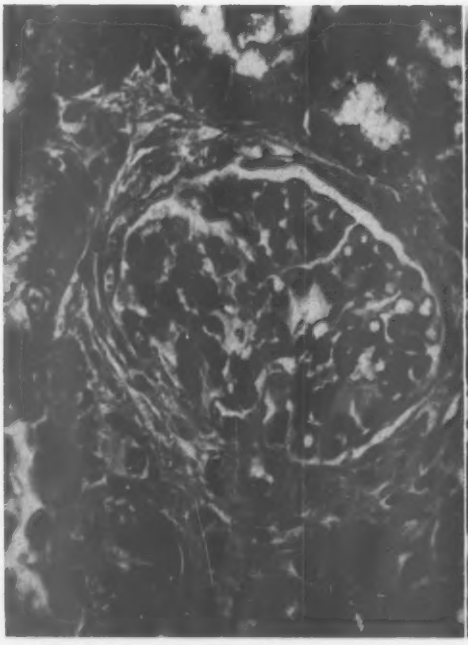
FIG. 12. Experiment 2. Eight weeks. Spontaneous death. Marked nodular glomerular hyalinization. × 404.

FIG. 13. Experiment 2. Eight weeks. Same field as that from which Figure 12 was made, showing hyaline thickening of capillary walls, nodular hyalin, and one hyaline arteriole between the two glomeruli. × 192.

FIG. 14. Experiment 2. Thirty-two weeks. Nodular hyalinization of glomerulus, thickened capillary walls, and periglomerular fibrosis. × 404.

FIG. 15. Experiment 2. Thirty-two weeks. Earlier stage of hyalinization. × 538.

FIG. 16. Experiment 2. Thirty-two weeks. Diffuse hyaline thickening of capillary basement membranes (membranous glomerulonephritis), and cellular increase about the glomerular stalk. × 404.



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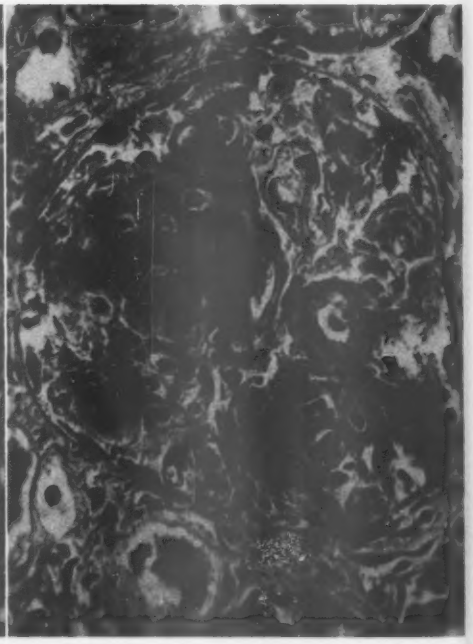

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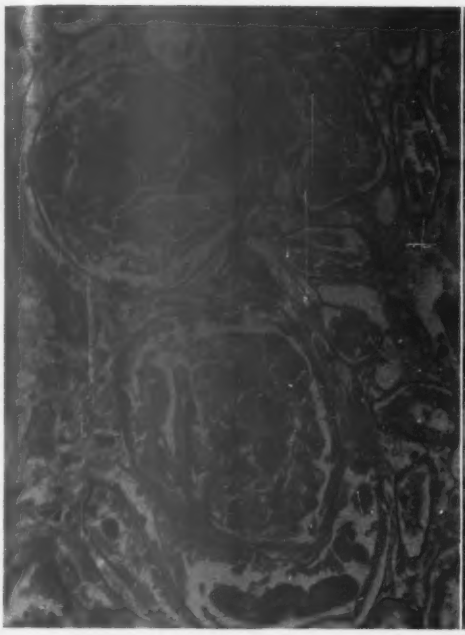

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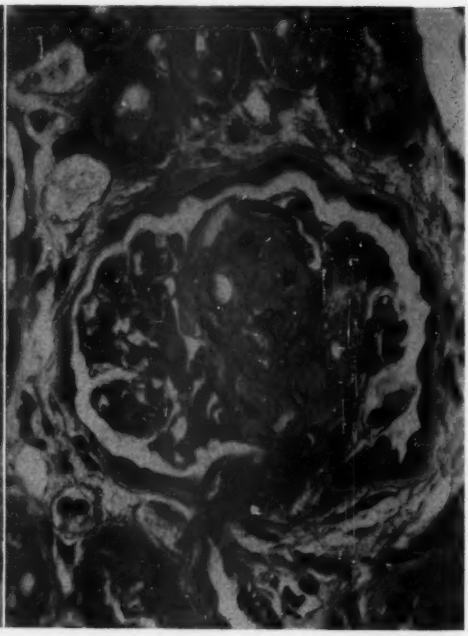

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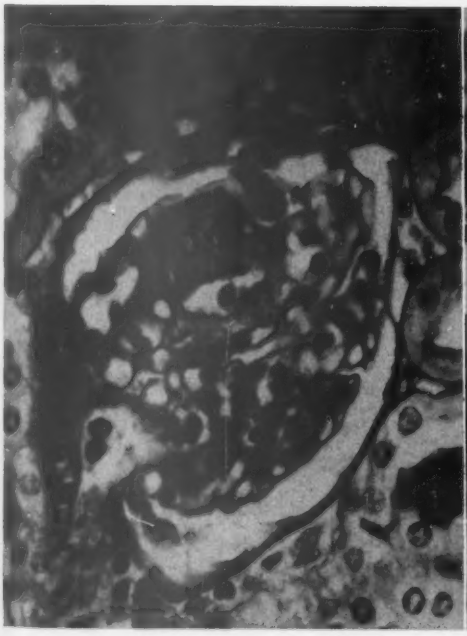

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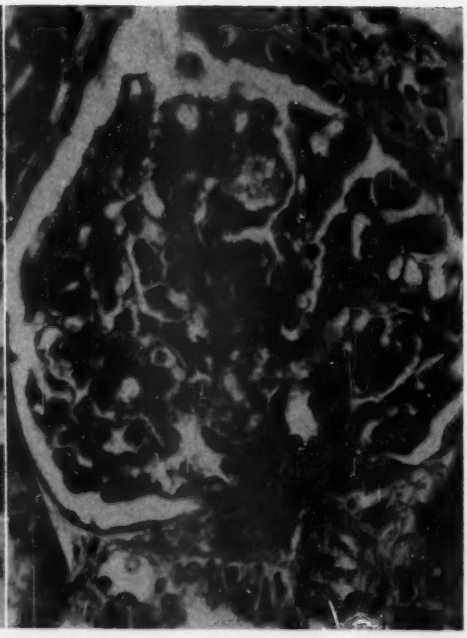

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INFLUENCE OF EXPERIMENTAL RENAL DAMAGE ON HISTOCHEMICALLY DEMONSTRABLE SUCCINIC DEHYDROGENASE ACTIVITY IN THE RAT *

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The abundance of various enzymes in the mammalian kidney is well recognized. In recent years methods have become available which permit the demonstration of some of these enzymes in tissue sections. Data have been published concerned not only with the distribution in the normal kidney, but also with the changes occurring under various experimental conditions. More recently techniques have been developed which permit the localization of dehydrogenase in certain structures of the nephron with either the help of tetrazolium salts[1] or potassium tellurite.[2] A further improvement in this technique was introduced by Seligman and Rutenburg,[3] making possible the histochemical localization of specific dehydrogenases, for instance, succinic dehydrogenase.

The present report deals with the changes that occur in succinic dehydrogenase activity in damaged and regenerating renal tubules. Since dehydrogenase activity has been associated with viability, it seemed of interest to determine whether loss of succinic dehydrogenase activity would precede the microscopic alterations in cells undergoing necrobiosis. The changes in dehydrogenase activity observed in regenerating cells and in those which have become atrophic due to experimentally induced hydronephrosis were compared with those in phosphatase activity under the same experimental conditions. Finally, a thorough study was made of the localization of dehydrogenase activity in the kidney of the rat and also of the rabbit, since conflicting statements have been found in the recent literature.[4-7]

MATERIAL AND METHOD

Young male rats of the Wistar strain, weighing 150 to 200 gm., were used for most experiments. The rats were kept on a complete Rockland diet. In order to study the possible influence of food and water, the kidneys of some rats were examined after the animals had been deprived of food or water for 48 hours. Some rats received intraperitoneal injections of 5 cc. of normal saline solution and were sacrificed 1 to 2 hours later.

* Received for publication, May 28, 1953.

For the induction of renal necrosis and the study of regenerating tubules, use was made mainly of dl-serine. This substance will invariably cause a reproducible degree of renal damage in the rat.[8] Dl-serine was given intraperitoneally in amounts of 1 mg. per gm. of body weight, dissolved in a few cc. of distilled water. The animals were sacrificed at stated intervals. In a few rats renal damage was induced with mercury. It has been previously determined that meralluride sodium* in amounts of 0.01 mg. of mercury per gm. of body weight, or approximately 2 ml. of a 1 to 50 dilution of the original preparation, will produce renal necrosis regularly in rats of 150 gm.[9]

For the production of hydronephrosis, larger rats weighing 200 to 350 gm. were used. The ureter of one kidney was ligated under light ether anesthesia and, at the time of sacrifice, both the ligated and non-ligated kidneys were removed for study. Parts of the kidneys were fixed in formalin for routine sections, while other portions were fixed in ice-cold acetone. These tissues were dehydrated with cedar wood oil and xylene, and paraffin sections were prepared for the demonstration of phosphatase activity.

Succinic dehydrogenase activity was demonstrated in fresh frozen sections cut routinely at 15 μ. For the study of its normal distribution in the kidneys of the rat and rabbit, sections were prepared which were only 5 to 10 μ thick. These sections were cut with a Sartorius microtome equipped with a special cooling device for the knife. This microtome was found to be very useful for the preparation of thin frozen sections suitable for histochemical staining.[10] In the earlier part of the study, the incubation mixture contained neotetrazolium and sodium succinate buffered at pH 7.4. Later we followed the suggestion of Padykula[5] and of Rutenburg, Wolman, and Seligman[6] and added calcium chloride and sodium bicarbonate to serve as activators. The solution used had the following composition:

0.2% neotetrazolium .....10 cc.	0.33 M calcium chloride.....0.2 cc.
0.2 M sodium succinate .....10 cc.	0.6 M sodium bicarbonate.....2.0 cc.
m/10 phosphate buffer, pH 7.4....10 cc.	Distilled water .....6.8 cc.

Sections were cut, dipped in tap water for a moment, and then immersed in the incubation mixture for 1 or 2 hours respectively at 37° C. under aerobic conditions. Longer incubation times did not increase the staining intensity. Sections were then washed in tap water, immersed in 10 per cent formalin overnight, mounted on glass slides, and covered with glycerin. The coverglass was rimmed with nail polish.

* Mercuhydrin sodium, Lakeside Laboratories, Inc., Milwaukee, Wisconsin.

Such sections, when kept in the dark, were usable for many months. With this technique, uniform and satisfactory staining was regularly observed. In view of the excellent results, it was not necessary to incubate sections under anaerobic conditions as suggested by others.[5,6] Occasional sections were counterstained with hematoxylin after they had been fixed in formalin.

Control sections were incubated in a solution which did not contain sodium succinate. As has been shown by Seligman and Rutenburg,[3] no staining is seen unless the incubation mixture contains a specific substrate, since endogenous dehydrogenase activity is destroyed in frozen sections. Of the various tetrazolium salts available at present, use was made of neotetrazolium chloride first investigated by Antopol and co-workers in this country.[1] In fresh frozen sections the colorless soluble substance is reduced to dark purple, water-insoluble formazan pigment in the presence of succinate. We had previously found this substance to be suitable for the demonstration of hydrogenases in white blood cells.[11]

Results

Distribution in the Normal Kidney

In the rat kidney enzymatic activity was found not only in the cortex but also in the outer portion of the medulla (Fig. 1). Sites of enzymatic activity were indicated by dust-like and coarse granules of purplish red reduced neotetrazolium. This pigment was often diffusely distributed throughout the cytoplasm but occasionally it showed a distinct localization around nuclei, particularly in certain portions of the rabbit nephron. The nuclei did not participate in the staining reaction. Glomeruli and blood vessels did not reduce neotetrazolium. However, occasional fine granules were seen in the walls of larger arteries. Within the cortex all portions of the tubular system showed prominent activity. Within the innermost or subcortical zone many tubular structures showed less intense staining (Fig. 2). In these the pigment appeared in the form of fine dust-like granules. The tubular components giving a less intense staining are obviously the distal portions of the proximal convolutions. They can be identified as such, since these are the same portions of the nephron which are selectively damaged by dl-serine, which acts only upon the distal portions of the proximal convolutions.[8] Some tubules located in the inner cortical zone, however, showed an intense staining reaction due to the deposition of coarse, purplish granules. A similar intense staining was seen in many tubules located within the outer medullary zone, obviously ascending or wide limbs of Henle's loops. The thin or descending limbs

of Henle's loops did not participate in the enzymatic reaction. The collecting tubules showed only a very slight reaction or none. The inner zone of the medulla, which contains thin loops of Henle and collecting tubules, showed almost no reaction. Only occasionally a few dust-like granules were identified in some of the collecting tubules. It is noteworthy that the staining intensity at the terminal portions of the proximal convoluted tubules differed considerably in various kidneys. In some the difference between the proximal and distal segments of the proximal convolutions was very marked. In others, however, the cells of the distal portions of the proximal convoluted tubules reduced neotetrazolium almost as intensely as those of the proximal portions.

Certain differences were found in the staining pattern of the kidneys of the rabbit and rat. Within the cortex of the rabbit kidney apparently all tubular structures reduced neotetrazolium as in the rat. However, the marked difference in staining intensity of proximal and distal portions of the convoluted tubules was not seen in the rabbit. Of all tubules of the rabbit, the most intense reaction was observed in ascending limbs of Henle's loops. In addition, scattered tubular segments found within the outer cortex showed the same intense staining as did thick limbs of Henle's loops (Figs. 3 and 4). The exact nature of these tubules could not be determined. There was considerable activity within collecting tubules located in the outer medulla. These structures were easily identified since medullary rays containing the collecting tubules are well developed in the rabbit kidney. Within these collecting tubules perinuclear localization of enzyme activity was quite prominent. Only little activity was found within the inner zone of the medulla. However, some of the collecting tubules showed such activity to a considerably more pronounced degree than was observed in the rat. Thin or descending limbs of Henle's loops showed no appreciable enzymatic staining.

The Influence of Deprivation of Water and Food and of Overhydration

Experiments were undertaken to determine whether the differences in stainability of the inner cortical zone could be influenced by deprivation of water and food or by overhydration. Starvation for 48 hours with and without access to drinking water had no influence. Similarly, overhydration by intraperitoneal injection of physiologic saline solution was without effect. Animals in these groups showed the same variation in staining intensitv in the inner cortical zone as did the nonfasted normal rats.

Influence of Dl-Serine

Acute Experiments. Rats given intraperitoneal injections of dl-serine were killed after 10, 20, 25, 30, 45, 60, 75, and 90 minutes, and after 6 and 24 hours. It had been shown previously that within 24 hours there occurred extensive necrosis of renal tubules within the subcortical zone. By isolating single units, the damage was found to be localized in the distal portions of the proximal convolutions. No other segment showed evidence of renal necrosis. It also had been reported previously that the first signs of renal damage would appear as early as 1 hour following an intraperitoneal injection of dl-serine.[8] In sections stained with hematoxylin and eosin, focal areas were noticed within the inner cortical zone in which damaged cells revealed eosinophilia while the nuclei were somewhat less intensely stained than those in uninvolved tubules. Occasionally the cytoplasm appeared shrunken or even fragmented. In damaged proximal convoluted tubules the brush borders often were separated from the eosinophilic tubular cytoplasm. Lesions of this kind were seen mainly in the kidneys of rats killed up to 3 hours after the injection. During subsequent hours diffuse necrosis became prominent.

The first changes in dehydrogenase activity became noticeable after 30 to 45 minutes. Some cells in tubular segments showed a brownish discoloration of the cytoplasm. Such abnormally staining tubular areas were widespread within the inner cortical zone after 60 to 90 minutes (Fig. 5). Within these abnormally staining tubular cells occasional coarse granules of reduced tetrazolium were still noticed. In contrast to undamaged cells, however, the fine dust-like deposition of formazan had vanished (Fig. 6). After 3 hours all of the tubules in the inner cortical zone showed these changes. After 6 hours and more the completely necrotic cells showed a reddish brown, diffuse coloration with little if any deposition of granular dye. The ascending Henle's limbs, which were not involved in the serine damage, retained their ability to reduce the neotetrazolium in the form of coarse granules, and these structures contrasted sharply with the necrotic tubules.

Regenerating Phase. Rats were killed 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 16, 20, and 26 days after the injection of dl-serine. Routine sections showed the well known microscopic appearance of tubular regeneration which became quite manifest after 48 hours. The necrotic epithelium was sloughed off and carried into the excretory tubules. Remnants of such necrotic epithelium were seen even after 2 weeks in occasional tubules. The denuded walls of the tubules were invested by proliferating cells which showed prominent nuclei and occasional mitotic figures.

The cytoplasm of this regenerating epithelium was scanty and the height of the cells was markedly reduced. This made the lumen of the tubules appear wider than normal. Even after 15 to 26 days, regeneration was incomplete. The epithelium, which by now lined all the tubules, had still not regained its normal appearance. In many places it was flat and lacked the eosinophilic staining properties so characteristic for the proximal convolutions. There was a moderate degree of interstitial inflammation and connective tissue proliferation. Calcific changes were not noticed.

In animals sacrificed after 48 hours, cells composing the necrotic tubules showed a brownish red tinge without deposition of any granular formazan. No enzymatic activity was noticed in the regenerating tubules up to the fifth day. After the fifth day evidence of dehydrogenase activity became demonstrable. In the cytoplasm of regenerating cells occasional dust-like granules were found scattered in the cytoplasm (Fig. 7). In the following days enzymatic activity in some of these regenerating tubules was seen to increase. There was, however, considerable variation in the amount of reduced neotetrazolium and even after 26 days some of the regenerating tubules lacked any dehydrogenase activity.

Observations made in sections stained for phosphatase activity confirmed results previously recorded.[12] In the acute phase of the intoxication, phosphatase was demonstrable in the necrotic tubules. In contrast, the regenerating epithelium revealed no appreciable enzymatic activity in animals which were allowed to live as long as 26 days following the administration of the injurious agent.

Experiments with Mercury. In these experiments rats were killed 24 and 48 hours after the administration of mercuhydrin. Tubular necrosis was widespread in the inner cortical zone. The microscopic changes observed in routine sections as well as the reduction of histochemically demonstrable dehydrogenase activity were similar to those seen in animals given dl-serine. There was no appreciable diminution of dehydrogenase activity in any other portion of the nephron.

Experimental Hydronephrosis. The kidneys in which the ureter was ligated showed the typical gross and microscopic changes, increasing in extent with the duration of the experiment. Grossly the kidney appeared enlarged and pale. On sectioning the renal pelvis was dilated. Under the microscope there was dilatation of the tubular system extending into the outer portion of the cortex. The capsular surfaces of the glomeruli were frequently distended. The epithelium in dilated tubules was flattened and in addition there was collapse of proximal

convoluted tubules. The extent of these changes varied considerably in different animals and even in different sections of the same kidney.

In animals sacrificed after 30 hours there was no significant change in dehydrogenase activity. Rats that were allowed to live for 6 to 8 days showed only very slight focal decrease of dehydrogenase activity. After 11 days, however, an appreciable depletion of enzymatic activity was found. Reduction of dehydrogenase activity was seen in dilated as well as in collapsed tubules (Fig. 8). The decrease of this enzymatic activity varied considerably and was only focal, even in kidneys which showed the most pronounced changes. In kidney sections of animals sacrificed after 11 days, considerable diminution of phosphatase activity was seen, as first described by Wilmer.[13] In general, depletion of phosphatase was much more marked. Many tubules were entirely devoid of activity. However, occasional tubules, even in the kidneys showing the most pronounced changes, still showed stainable phosphatase.

Comment

Malaty and Bourne[4] found the distribution of dehydrogenase activity similar to that of phosphatase in kidneys of various mammalian species, since stainable dehydrogenase was present in the cortex but almost none in the medulla. Rutenburg and his co-workers[6] found maximum dehydrogenase activity in the descending loops of Henle, and less in distal and proximal convolutions. The epithelium of ascending limbs of Henle's loops reacted least intensely. These authors worked with mouse, rat, hamster, guinea-pig, dog, and rabbit. Shelton and Schneider[7] used tissue blocks of mouse kidneys and incubated them in a mixture which contained sodium succinate. In frozen sections cut from these blocks, activity was demonstrable in proximal and distal convoluted tubules and in Henle's loops and, to some degree, in collecting ducts. The papilla was only slightly stained. Padykula[5] found the cortex and outer two thirds of the medulla highly active in the rat kidney. Succinic dehydrogenase activity was present in greatest concentration in the initial portion of the proximal convoluted tubules and in the tubules of the mid-region of the medulla, apparently localized in both loops of Henle and in collecting ducts. The inner cortical zone, containing both the terminal portions of the proximal convoluted tubules and portions of the loops of Henle, stained more lightly.

Our results in the rat are in general agreement with the findings of Padykula,[5] although they differ in some details. We found in both the rat and rabbit, activity localized not only in the cortex, as stated by

Malaty and Bourne,[4] but also within the outer medullary zone. Faint activity in some tubules was noticed also in the inner medullary zone. In both species proximal and distal convolutions were active. In the rat, as described by Padykula, the terminal portions of the proximal convoluted tubules often showed less activity than the proximal portions. This, however, was not the case in the rabbit. In contrast to the rat, occasional tubules that could not be identified in the cortex of the rabbit kidney showed very heavy formazan deposition. The thin or descending limbs in both species showed apparently no significant activity while the broad or ascending limbs showed heavy formazan deposits. In both species the initial portion of the collecting tubules located within the cortex proper were stained. The collecting tubules in the outer medullary zone of the rabbit kidney, which are easily identified by their location in medullary rays, show formazan deposition. In the rat, however, these structures are apparently not regularly stained. Within the inner medullary zone there is moderate activity in large collecting ducts in the rabbit and only very little and occasional activity in the rat.

It is admittedly very difficult to identify the various portions of the nephron in microscopic sections, particularly in those in which cellular details are obscured by deposits of granular dye. The only satisfactory technique for the localization of functional processes in certain parts of the renal tubules consists in the isolation of single renal units, as pointed out by Oliver,[14,15] who, several years ago, attempted the maceration of rabbit kidneys that had been stained for unspecific dehydrogenase. However, the tissue became so brittle after maceration that no satisfactory results were obtained. Nevertheless, careful study of thin sections will permit the correct identification of most renal tubules. The fact that in the rat kidney dl-serine affects the terminal portions of the proximal convolutions exclusively is helpful in identifying these segments. Some of the discrepancies in the literature may be accounted for by the examination of much thicker sections than were used in the present study. As is the case with other enzymes demonstrable with histochemical techniques, considerable variations apparently exist in the distribution of dehydrogenase activity in the same organs of various species. One should, therefore, be careful not to draw too far-reaching conclusions as to the possible function of an enzyme found in certain localizations unless one is sure that such a localization is consistently present in various species.

Contrary to the opinion of Malaty and Bourne,[4] there is a very marked difference in the distribution of alkaline phosphatase and

dehydrogenase activity, since alkaline phosphatase is limited to tubular segments located in the cortex of the kidney. Although some investigators found all portions of the nephron within the cortex of the rat to contain stainable phosphatase,[16] we have found this enzyme exclusively in the proximal convoluted tubules. This was observed not only in paraffin sections but also in thin, unfixed, frozen sections which were incubated for 15 minutes. The use of unfixed tissues prevents the possible destruction of enzymatic activity by dehydration and paraffin embedding.[10]

No obvious reason could be found for the variability in dehydrogenase activity in the terminal portion of the proximal convoluted tubules in the rat kidney. Neither the withdrawal of food or water nor overhydration seemed to be of importance. It is of interest that this segment of the renal unit is particularly vulnerable to toxic agents in the rat. Certain substances, such as dl-serine[17] and ethionine,[18-20] act exclusively on this portion of the nephron in the rat but not in other animal species so far tested.

Originally it was thought that the tetrazolium salts could be reduced only by living tissue. On this assumption they have found wide acceptance as a means of determining germinating ability of various seeds.[21] Later, however, it was discovered that tissue extracts under favorable conditions also can reduce tetrazolium salts.[22] The enzyme system, however, is very sensitive to physical and chemical insults. This is demonstrated, for instance, by the fact that frozen sections prepared from fresh tissues lose their endogenous hydrogenase activity, as previously mentioned. The reduction of tetrazolium salts by enzymes of living tissue cannot be considered as a general test of life. Nevertheless, differences in tissue viability may be detected by their use.[23] It was thought possible that cells which were in the process of undergoing necrosis might reveal loss of dehydrogenase activity before microscopic changes indicating impending cell death were discernible by routine staining techniques. These expectations were not entirely fulfilled since evidence of necrosis was seen in routine sections almost simultaneously with changes in dehydrogenase activity. However, changes in enzymatic staining far exceeded, in most instances, those seen when only routine staining was employed. Once necrosis had set in, dehydrogenase activity completely disappeared, although necrotic cells still took on a diffuse brownish color without deposition of the typical granular deposits. It has been suggested[3,23a] that such an offshade diffuse discoloration of the cytoplasm may be due to only partial reduction of tetrazolium salts, indicating the presence of a lesser de-

gree of enzymatic activity. However, one must also take into consideration the fact that cells undergoing necrobiosis are stained diffusely by certain dyes.[24] It seems possible that the necrotic cells take up traces of reduced neotetrazolium formed in other locations with high dehydrogenase activity. This possibility was suggested by the behavior of the enzyme in regenerating epithelium. In such cells dehydrogenase activity was indicated by distinct isolated granules which were present only in small numbers and diffuse cytoplasmic staining, comparable to that seen in necrotic cells, was not observed. Reappearance of stainable dehydrogenase took place after the fifth day but was incomplete even after 26 days. In the hydronephrotic kidney disappearance of dehydrogenase activity was slow and incomplete.

In comparing the behavior of dehydrogenase activity and that of phosphatase and esterase it was obvious that the reduction of the hydrolytic enzymes was considerably more distinct and extensive. Similarly, the regenerating tubules showed almost no phosphatase activity up to 26 days. In contrast, necrotic cells retained their phosphatase activity.

Zweifach, Black, and Shorr[25] described the patterns of formazan deposition in the proximal convoluted tubules with striking differences between normal and hypertensive kidneys obtained from dog, rat, and man. They used sections cut from slices that had been incubated in a mixture containing tetrazolium chloride and sodium succinate. While in the normotensive kidneys the dye was distributed in the form of fine dust-like granules, in hypertensive kidneys it appeared as coarse clumps, plaques, or needles. In none of the experimental conditions reported in this paper was a similar phenomenon observed.

Succinic dehydrogenase activity can be found in many locations. It is particularly plentiful in the kidneys, liver, and heart of various mammalian species.[6] In these organs high metabolic activity is apparently closely related to the presence of succinic dehydrogenase activity. It is interesting to note that under certain conditions the amount of stainable dehydrogenase will increase in cells which ordinarily show only a little. The granulosa cell layer of the cystic follicles of the rabbit ovary, for instance, shows little succinic dehydrogenase activity. After the follicles have been stimulated with the hormones contained in the urine of pregnant women, their enzymatic activity is markedly increased.[26] In human blood cells the young myeloid cells show no stainable dehydrogenase while polymorphonuclear leukocytes become positive.[11] It also has been demonstrated that the functional activity

of adrenal cortical cells is apparently paralleled by comparable changes in dehydrogenase activity.[27]

Succinic dehydrogenase is an important link in the chain of enzymes which are responsible for biologic oxidation. It is one of the oxidative steps necessary for esterification of inorganic phosphate in energy-rich form.[28] It seems probable that its presence in most of the tubular components of the renal nephron is connected with the provision of energy for the proper function of these renal components. It is interesting to note that the hydrolytic enzyme phosphatase is not inactivated in necrotic cells while succinic dehydrogenase is. Unless cells are completely destroyed, however, the renal tubules will retain succinic dehydrogenase activity more avidly than phosphatase. The former will also reappear earlier than phosphatase when destroyed tubular cells are regenerating. It is apparent, however, that cellular regeneration will commence days before succinic dehydrogenase activity becomes demonstrable. Succinic dehydrogenase activity in the renal tubules is apparently primarily connected with the proper function of the cells. The considerable degree of preservation of dehydrogenase activity in the hydronephrotic kidney is in good agreement with the well known fact that these kidneys retain some functional activity for a long time, as evidenced, for example, by their ability to concentrate certain dyes.[29]

Summary

The distribution of succinic dehydrogenase activity was studied in thin frozen sections of the kidney of the normal rabbit and rat, and of the rat kidney under various experimental conditions. All tubular segments of the nephron located in the cortex were found to be active. Within the outer medulla, collecting tubules of the rabbit showed considerably more activity than those of the rat while the ascending limbs of Henle's loops showed marked activity in both species. In the kidney of the rat the terminal portions of the proximal convoluted tubules showed appreciably less dehydrogenase activity than the proximal portions. This was not seen in the rabbit. In the latter species, however, some unidentified tubular segments localized in the cortex showed stronger staining than the surrounding proximal convoluted tubules.

In tubules made necrotic by either dl-serine or mercuhydrin the normal staining pattern disappeared rapidly. When dl-serine was given, evidence of altered staining was seen after 45 to 60 minutes. Regenerating tubules revealed evidence of enzymatic activity after 5 days. However, even after 26 days a normal enzymatic staining pat-

tern was not seen. In experimental hydronephrosis there occurred only moderate diminution of enzymatic activity up to 11 days.

In contrast to the behavior of dehydrogenase activity, phosphatase remained active in necrotic cells. It was not demonstrable in regenerating cells up to 26 days. In experimental hydronephrosis this enzyme disappeared at a faster rate and to a significantly more marked degree than did succinic dehydrogenase activity.

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[*Illustrations follow*]

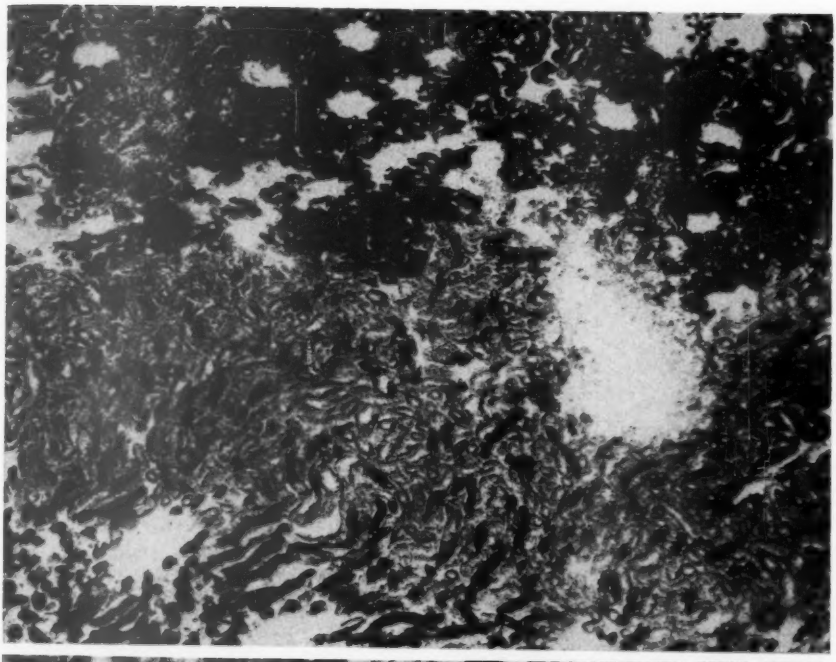
LEGENDS FOR FIGURES

All photomicrographs are from fresh frozen sections cut at 10 to 15 μ and prepared for the demonstration of succinic dehydrogenase activity without counterstain.

FIG. 1. Cortical and subcortical portions of the kidney of a normal rat. The terminal portions of the proximal convolutions in the subcortical zone show less enzymatic activity while the ascending limbs of Henle's loops are stained as intensely as the convoluted tubules in the outer renal cortex. × 75.

FIG. 2. A higher magnification of a portion of the section shown in Figure 1. × 150.

1



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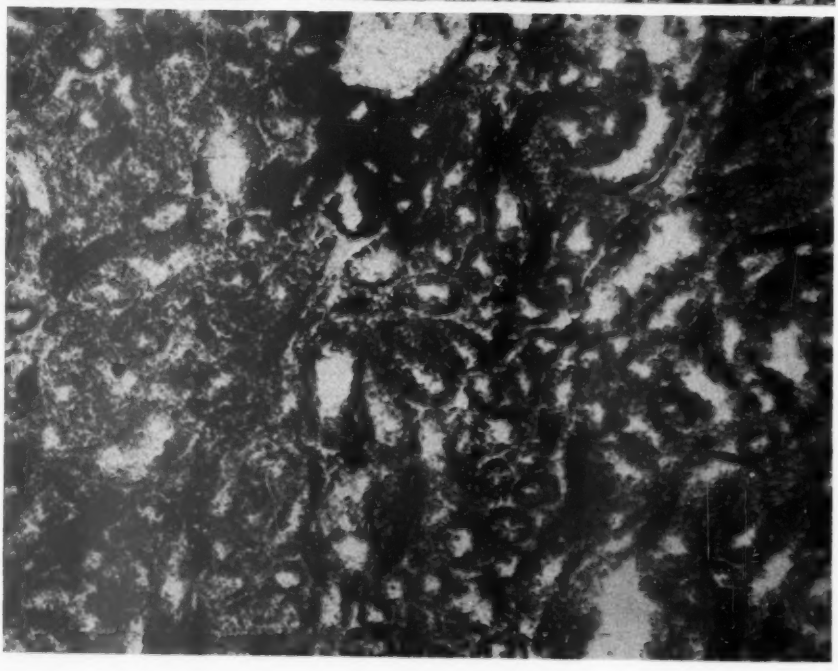
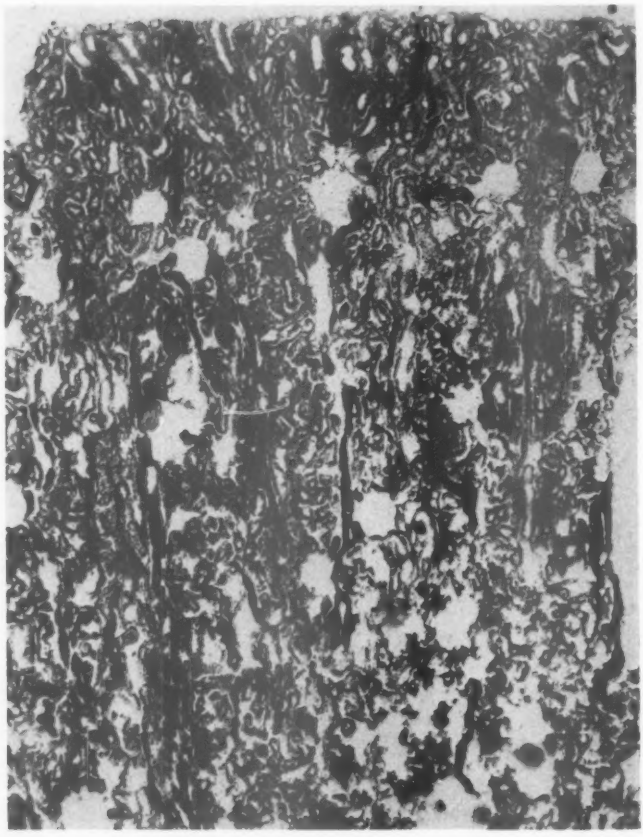


FIG. 3. Cortical and subcortical portions of a normal rabbit kidney. All tubular structures show enzymatic activity. Occasional tubules not clearly identified show a very strong staining reaction. × 75.

FIG. 4. A higher magnification of a portion of the section shown in Figure 3. The subcapsular portion of the cortex is at the right. × 150.

FIG. 5. Rat kidney. Animal sacrificed 90 minutes after the intraperitoneal administration of dl-serine. There is considerable reduction of dehydrogenase activity in many of the tubules located in the subcortical zone. Ascending limbs of Henle's loops which are not involved retain their prominent staining reaction. × 75.



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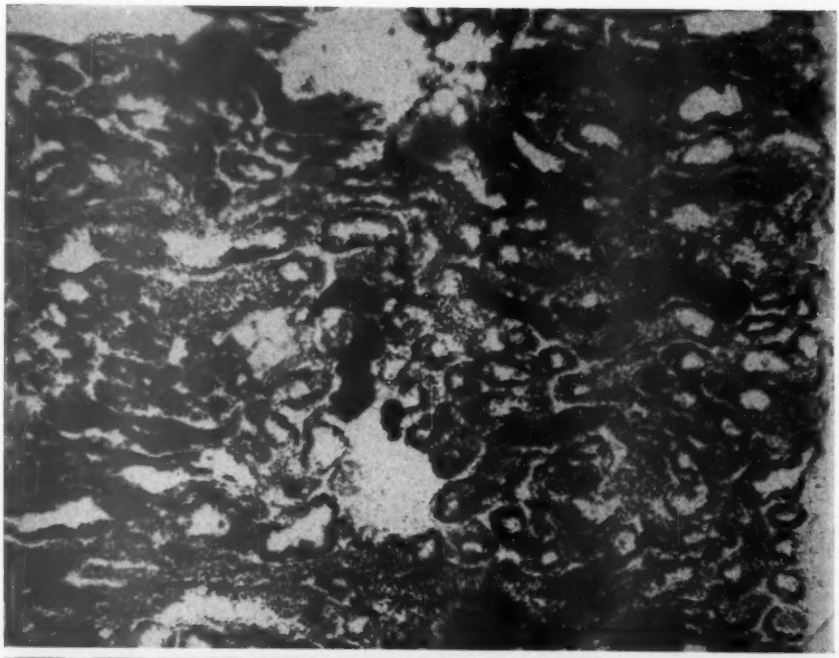

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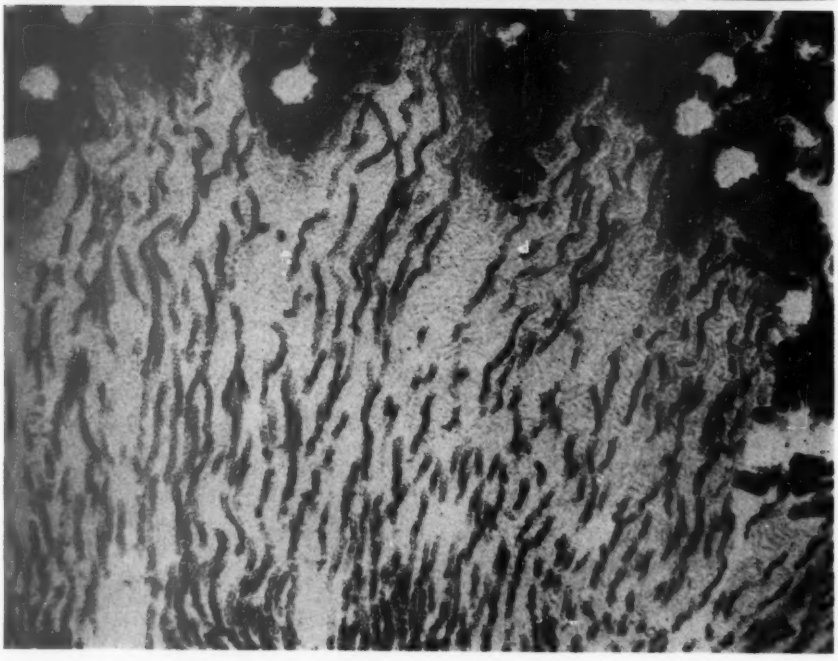

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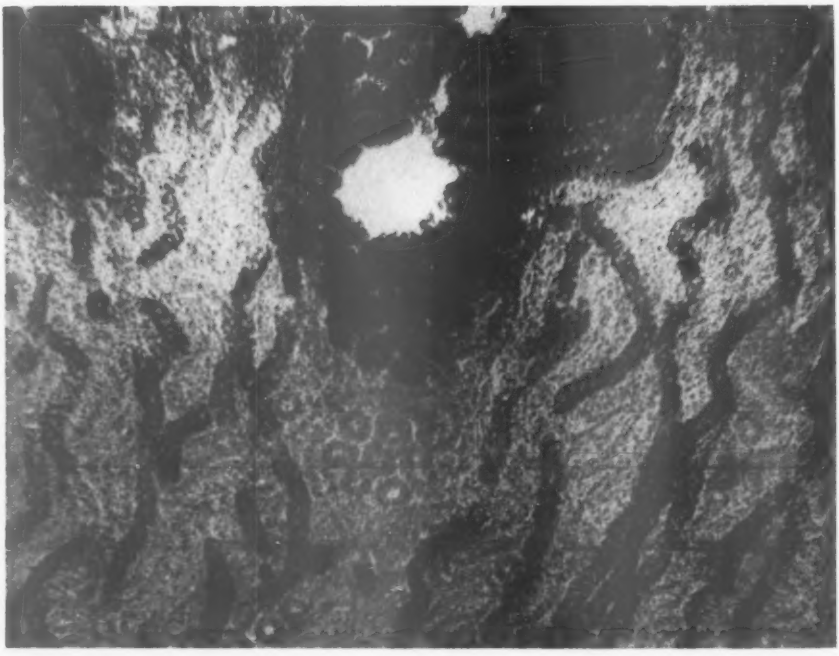
FIG. 6. A higher magnification of a portion of the section shown in Figure 5. × 150.

FIG. 7. Rat kidney. Animals sacrificed 6 days after the intraperitoneal administration of dl-serine. There is slight enzymatic activity in the regenerating tubules. The cortical portion is at the right. × 150.

FIG. 8. Rat kidney. Animal sacrificed 11 days after ligation of the ureter. There is moderate diminution of enzymatic activity in dilated and collapsed cortical tubules. The renal capsule is shown at the right. × 150.



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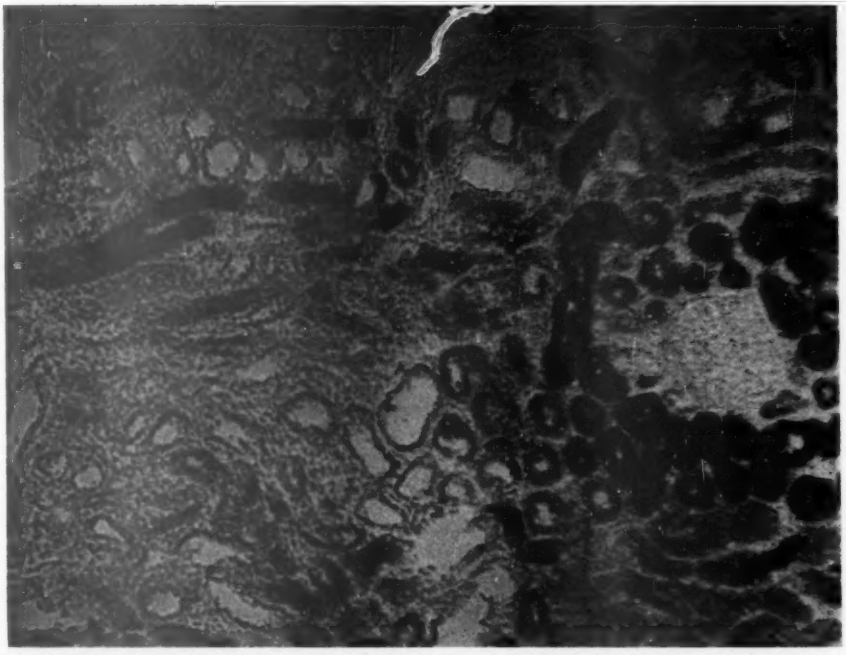

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COR PULMONALE IN MANSON'S SCHISTOSOMIASIS

I. Frequency in Necropsy Material; Pulmonary Vascular Changes Caused by Schistosome Ova *

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Some patients with late schistosomiasis, in the stage of hepatic cirrhosis, eventually present a clinical picture of chronic cor pulmonale with congestive failure, due to involvement of the small pulmonary arteries. The purpose of this first paper is to report the frequency of cor pulmonale on this basis in our necropsy material, and the microscopic changes caused by schistosome ova in small branches of the pulmonary artery. A review of the literature on the pulmonary vascular alterations in schistosomal cor pulmonale is also included.

Studies on the histopathologic changes of the pulmonary arterial tree in schistosomiasis are few, and most of them are perfunctory. One of the first reports is that of Miller[1] (1914) whose patient presented in the lung numerous schistosomal granulomata or pseudotubercles,[2] and a fibrous thickening of the larger and smaller branches of the pulmonary artery with a tendency to obliteration of the smaller ones. Sorour[3,4] (1928, 1930), describing the vascular lesions in schistosomiasis, with an occasional reference to the pulmonary vessels, reported changes due to ova and dead worms, the former causing endothelial proliferation with occlusion of the lumen, or parietal granulomata; the worms stimulated endothelial proliferation only. Endothelial proliferation could be elicitated also through the action of toxins alone.[4] In the case reported by Clark and Graef[5] (1935) there were schistosomal granulomata originating mainly in the pulmonary arteries, with obliteration of lumina by a richly vascularized tissue. Such arterial obliteration would produce pulmonary hypertension, which could then be responsible for intimal connective tissue thickening found in small pulmonary arteries. Day[6] (1937) did not present the histopathologic findings in his case, but stated that they were similar to those described by Clark and Graef.

Shaw and Ghareeb[7] (1938), in an excellent paper, presented cases of focal (six) and widespread (six) arterial lesions, the latter produc-

* Received for publication, June 4, 1953.

Portions of this material have appeared previously in a thesis of limited distribution by the same author: Histopatologia da endarterite pulmonar esquistossomótica. Emprêsa Gráfica da "Revista dos Tribunais" Ltda., São Paulo, 1952.

ing hypertrophy and dilatation of the right ventricle of the heart, with congestive failure. Lesions of both types were caused by direct action of ova, attacking a few arterioles in the focal type and numerous arterioles in the diffuse type. In the focal lesion, the ovum, as embolic material, was impacted in the arteriolar lumen and produced arteriolitis necroticans with necrosis of the inner layer and of the media, without thrombosis and aneurysm formation. A reparative process then followed, as a result of which arteriolitis obliterans appeared, formed by a focal fibrocellular thickening of the inner coat with narrowing or occlusion of the lumen. Frequently, the occluding tissue became canalized and the new vessels dilated, producing a so-called angiomatoid structure in which the media was deficient or absent. A granuloma was formed only when the ovum attained an extravascular position. The widespread arterial lesions were essentially the same as the focal ones, but diffusely scattered due to the high number of ova, and new lesions were superimposed on old. As a consequence of the arteriolar obstruction, the larger arteries presented thickening of the intima, hypertrophy of the media, and collagenous thickening of the adventitia.

Jaffé[8-11] (1939, 1940, 1944, 1948) reported endarteritis and periarteritis in schistosomiasis, without mentioning the number of cases in which the pulmonary vessels were involved in association with pulmonary hypertension. The picture of both processes was described briefly as a fibrous thickening and round cell infiltration of the inner and outer coats respectively. Jaffé believed that these vascular lesions were not due to the direct action of the ova or worms but to circulating toxins,[8-10] later[11] (1948) invoking an allergic mechanism. The simultaneous involvement of arteries and veins of different organs without ova or worms, as, for instance, lungs, heart, and spleen, was mentioned by Jaffé as supporting the theory of the toxic origin of the vascular lesions. In addition Jaffé[10,11] described granulomata originated in blood vessels by impacted ova, producing a picture of circumscribed nodose panvasculitis.

Koppisch's case[2] (1941) presented ova in the wall of branches of the pulmonary artery with fibrosis of the intima and obliteration of the lumina. The histopathologic description of Potenza[12] (1943) on 3 cases was short, without reference to the necropsy findings of the heart, and his conclusions were essentially similar to those of Jaffé.

Cases with diffuse arterial lesions, similar to those reported by Shaw and Ghareeb,[7] have been presented by others.[13-15] In the case of Popper and Volini[16] (1949), with cor pulmonale of problematic etiology, there was, in branches of the pulmonary artery, a fibrous thickening of the wall and acute lesions suggesting periarteritis nodosa. The myo-

cardium of this patient, however, showed periarterial scars apparently developed from Aschoff nodules, and slight active arteritis. Meira, Behmer, and Bloise[17] (1951), in the case included in this study, observed a thickening of the intima of the pulmonary arterioles with preservation of the internal elastic membrane. Recently Girgis and collaborators[18] (1953) published a short histopathologic study of 2 cases, without new microscopic findings.

Other changes described in schistosomal lungs are outside the scope of this paper and need not be reviewed here (Mainzer[19]). Ova, with or without granuloma formation, have been reported in perivascular or peribronchial connective tissue, alveolar septa, or alveolar lumina,[2,7,10] the last two locations being parenchymatous granulomata.[7]

Material and Methods

In the necropsy registry of this Department, between 1937 and 1952 (necropsy numbers 8,899 to 35,000), there are 180 cases in which Manson's schistosomiasis was present either as the main disease or as a secondary necropsy finding. Of the 180 cases, 89 were in the stage of hepatic cirrhosis; of these 66 were males. In the cirrhotic group, 12 patients showed hypertrophy and dilatation of the right ventricle of the heart due to pulmonary arterial changes; the left cavities were normal. Ten (nos. 1 to 10) of the latter cases are presented here.* Two or three (usually three) blocks were taken, at random, from the lungs of each of the 10 cases. These measured between 1.0 by 1.0 cm. and 2.0 by 1.7 cm.; commonly they were of the larger sizes. As controls, one or two pieces of lungs of 8 patients (cases 11 to 18) with schistosomal hepatic cirrhosis were taken for examination, all of which presented esophageal varices. The material was fixed in 10 per cent formalin and embedded in paraffin. All blocks were cut serially, except those of case 8 and control cases 11 to 15, the sections being 6 μ thick. Of the blocks of case 8 and control cases 11 to 15 only some sections were examined, as were deemed necessary. In addition, frozen sections were made. Staining methods used were hematoxylin and eosin, Weigert's elastic tissue and van Gieson's stains, Perdrau's reticulin and van Gieson's stains, Mallory's aniline blue, Weigert's fibrin, Mallory's phosphotungstic acid hematoxylin (without chromium mordanting), Hotchkiss' periodic acid leukofuchsin,[23] cresyl violet, crystal violet, toluidine blue, techniques for iron (Perls's), and for lipids (scarlet red, Fischer).

* Five of these cases were reported, with a brief histopathologic study of the pulmonary vessels in 4 cases [14,17,20,21]; the fifth was the subject of a publication on schistosomal myocarditis,[22] but without histologic study of the pulmonary vessels.

The microscopic examination of the sections was as exhaustive as possible in regard to the pulmonary vessels. The vascular diameter was measured, including the adventitia. Sections of both ventricles of the heart, of the liver, and sometimes of other organs also were examined.

The name arteriole is employed for a vessel provided with all layers, in which the internal and external elastic membranes are apparent and the media is formed by a single row of cells; precapillary is used for a vessel without a media, having therefore both elastic membranes united.[24,25] The diameter of the pulmonary arterioles is considered to vary between 40 and 90 μ,[25] and that of the precapillaries between 15 and 30 μ.[24] In this report arteries of muscular type, possessing more than one row of muscle cells and between 100 and 200 μ in diameter, will be referred to as small arteries; larger arteries are those of muscular type with a diameter greater than 200 μ. Pulmonary parenchyma is used here to designate the functional part of the lung (from the respiratory bronchioles on), plus the immediate connective and vascular framework, named "unit of the lung" by Maximow and Bloom.[26]

Results

Ten of our 180 necropsy cases of schistosomiasis (5.5 per cent) presented verified schistosomal cor pulmonale. (Two additional cases were not studied histologically and are not included in this study.) This percentage becomes even higher (11.2 per cent) if only the 89 cases presenting schistosomal liver cirrhosis are considered. Nine of the 10 patients with cor pulmonale were female (90.0 per cent) and most of them were young (Table I).

Table II presents the main necropsy findings. Grossly, the lungs showed chronic passive congestion; the pulmonary arterial tree presented a diffuse thickening of the walls of the small branches; and, in a few cases, atherosclerosis (cases 1, 2, and 9) and intimal lipoidosis (case 10) were found in the larger branches. Schistosomal nodules (minute and whitish) were seen only in cases 8 and 10; case 8 presented also thin fibrous cords in the bases and posterior margins of both lungs. The thickness of the right ventricle varied between 0.6 and 1.5 cm. (Figs. 1 and 2).

Microscopic Examination

Lungs. Cases of Cor Pulmonale (Nos. 1 to 10)

Lesions Due to Schistosome Ova

Carried by the pulmonary arterial blood, the ova were retained in the small pulmonary arteries, or, much more infrequently, in the pulmonary arterioles, precapillaries, and larger arteries (above 200 μ in

TABLE I

Clinical Data in Cases with (Nos. 1 to 10) and Without (Nos. 11 to 18) Schistosomal Cor Palmonale

Case no.	Initials	Sex	Race	Place of birth (States of Brazil)	Age	Cardio-respiratory manifestations										Genito-urinary manifestations			Laboratory examinations									
										2nd sound in pulmonary area									Blood cells									
																					Differential count							
						Time of onset	Dyspnea	Cyanosis	Stasis of jugular vein	Accentuation	Reduplication	Palpitation	Edema of lower limbs	Arterial blood pressure	Pulse rate	Oliguria	Albuminuria	Amenorrhea	Red count	Total white count	Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes	Hemoglobin	Wassermann test	Kahn test
					yrs.	*mos.*								*mm. Hg*					*per cmm.*	*per cmm.*	%	%	%	%	%	%		
1	N.B.	F	W	Pernambuco	25	5	+		+	+	+	+	+	100/90	128	+	+											
2	M.G.A.	F	W	Sergipe	33	1½	+	+	+	+			+	115/80		+	+		3,250,000	7,200	66	4	0	25	5	79		
3	J.B.S.	M	W	Baía	25	5	+				+*	+		110/74	88		+		3,970,000	2,670	53.5	5.5	0.5	32.5	8.5			
4	A.T.S.	F	Ml	NE Brazil	20	1	+†						+	106/50	112	+		+										
5	M.L.M.	F	Ml	Alagoas	24	20 days	+						+‡	110/75	100		+										–	–
6	M.D.O.	F	W	Minas Gerais	29					+	+							+	4,200,000	5,000	51	18	1	28	2	74		
7	M.C.B.	F	Ml	Minas Gerais	40	6	+	+	+	+	+	+	+	140/70	70			+	3,700,000	9,700	41	4	0	53	2	61	–	–
8	M.A.S.	F	W	Alagoas	18																							
9	I.B.S.	F	W	?	18																							
10	B.F.C.	F	W	Pernambuco	30	12	+	–	–	+	+	+		100/60	104	–	–		1,800,000	8,300	56	3	0	36	5	33		
11	S.L.S.	M	W	Baía	22									120/70					4,200,000	3,200	75	7	1	14	3	90		
12	A.A.	M	W	Baía	17									80/0														
13	A.B.S.	M	W	Pernambuco	17								+	110/65	112				3,500,000	4,000	65	12	0	18	5		–	–
14	H.T.	M	W	Alagoas	28									120/40					1,600,000	9,300	85	0	0	12	3			
15	I.S.S.	F	W	Pernambuco	26									100/70				+	3,400,000	7,400	46	38	2	12	2	58	–	–
16	L.C.L.	M	W	Sergipe	15								+	120/60														
17	E.A.S.	F	W	Alagoas	36									90/50	132				3,900,000	12,400	68	3	1	21	6	50		
18	A.T.C.	M	W	Alagoas	33		+						+	115/70	108				1,700,000	4,500	81	1	0	17	1	30		

Ml. = mulatto.
Patient no. 7 presented sickle cell anemia.
* Reduplication in mitral area.
† Orthopnea.
‡ Anasarca.

TABLE II

Pertinent Necropsy Findings for the Present Study. Cases 1 to 10 with Schistosomal Cor Pulmonale; Others Are Controls

Case no.	Necropsy no.	Hours after death	Hours preserved in icebox	Weight	Stature	Transudations				Lungs			Heart									Aorta	Esophageal varices	Liver			Splenic weight (chronic splenomegaly)	Cause of death
						Right pleura	Left pleura	Pericardium	Peritoneum	Passive hyperemia	Small pulmonary arteries	Schistosomal nodules	Weight	Circumference of valves				Valvular and parietal endocardium	Thickness of right ventricle	Thickness of left ventricle	Dilatation of right ventricle			Weight	Passive hyperemia	Schistosomal cirrhosis		
														Tricuspid	Pulmonary	Mitral	Aortic											
				kg.	*cm.*	*ml.*	*ml.*	*ml.*	*ml.*				*gm.*	*cm.*	*cm.*	*cm.*	*cm.*		*cm.*	*cm.*				*gm.*			*gm.*	
1	27,028	7	N.P.	40	152	200		180	4,000	+	Thickened	o	300	11.5	6.5	8	6	N	0.7	0.8	+	N	+	1,800	+	+	240	R.H.F.
2	20,387	9	N.P.	58	148	700	105		3,000		Thickened	o	320	11		N	N	N	0.7	1.0	+	N		2,720	+	+	250	R.H.F.
3	23,364	28	3	52	165	o	o	o	o	+	Thickened	o	400	10.5	7	8	6	N	0.8	1.4	+	N	+	1,500	o	+	740	Hem.
4	27,767	12	5	42	155	o	o	o	200	+	Thickened	o	360	9.5	7	8.5	6.5	R.A.T.	0.8	1.2	+	N		1,600	+	+	600	R.H.F.
5	29,862	50	5	60	160	o	o	o	o	+	Thickened	o	470					N	0.8	1.2	+	N		1,400		+	500	R.H.F.
6	31,423	17	N.P.	60	175	o	o	o	o	+	Thickened	o	200	12	7	8	6.5	N	0.8	1.2		N		1,200	+	+	1,000	Hem.
7	31,493	15	N.P.	53	165	o	o	o	o	+	Thickened	o	340	10.5	7.5	8.5	6.5	T.M.V.	0.7	1.5	+	N			+	+	Agenesia	Bp.
8	10,780	31			145	1,000	1,000	o	2,000	+	Thickened	+						N	1.5	N	+	N	+		+	+		R.H.F.
9	34,161	5	N.P.	?	?	o	o	300	400	+	Thickened	o	300	10	5	7	4	N	1.0	1.0	+	N		1,700	+	+	450	R.H.F.
10	34,833	42	21	59	155	300	80	40	600	+	Thickened	+	300	11	8	9.5	6	N	0.6	0.9	+	N	+	1,250	+	+	O.S.	R.H.F.
11	26,116	30		52	160	o	o	o	o	o			250	10.5	6	9	5	N	0.4	1.3	o	N	+	1,300		+	860	R.E.V.
12	24,356	31	5	38	160	o	300	o	300	o			240					N	0.5	1.0	+†	N	+	1,250	o	+	580	R.E.V.
13	30,291	37	24	30*	150	o	o	o	2,000	o			140	N	N	N	N	N			o	N	+	900	o	+	980	R.E.V.
14	30,925	14	N.P.	64	165	200	o	o	3,000	o			280	12	8	10	7	N	0.3	1.4		N	+	1,820	o	+	1,580	R.E.V.
15	31,557	25	21	50	163	o	o	o	300	o			220	11	7.5	10	7	N	0.4	1.7	?	N	+	1,600	o	+	1,324	R.E.V.
16	32,740	54	48	43	150	o	o	o	200	o			200	10.5	8	8.5	6	N	0.3	1.3	o	N	+	1,110	+	+	580	G.I.H.
17	32,744	42	19	52	155	Scanty	Scanty	o	2,800	o			220	10.5	8	8.5	7	N	N	N	o	N	+	940	o	+	O.S.	C.H.
18	33,601	31	25	70	170	o	o	o	3,400	o			350	11.5	7.5	9.5	6.5	N	N	N	o	N	+	1,500	o	+	1,450	Hem.

Bp. = bronchopneumonia; C.H. = Coma hepaticum; G.I.H. = gastro-intestinal hemorrhage; Hem. = hematemesis; N. = normal; N.P. = not preserved; O.S. = old splenectomy; R.A.T. = right atrial thrombosis; R.E.V. = rupture of esophageal varices; R.H.F. = right heart failure; T.M.V. = thickening of free margins of the mitral cusps.

* Somatic and genital underdevelopment.

† All heart cavities were dilated due to diffuse chronic myocarditis.

diameter). Ova might be found, with or without inflammatory reaction around them, in the lumina of these vessels, or retained in the walls while passing through them, or in an extravascular location. The ovum induces the formation of a granuloma or pseudotubercle, the structure of which is well known.[2] In these granulomas there were many, few,

TABLE III

Microscopic Changes in the Lungs Due to Schistosome Ova. Cases 1 to 10 of Cor Pulmonale Group; Cases 11 to 18 Without Cor Pulmonale

Cases	Calcified ova without inflammatory reaction	Schistosomal granulomas	Scars of parenchymatous granulomas	Destruction of the wall of pulmonary arterial branches			Arteriovenous aneurysms	Ova without inflammatory reaction and lesions caused by ova; area of each section, about 2.5 sq. cm., thickness, 6μ
				Focal	Diffuse	Pseudo-aneurysms		
								total number
1	o	++	++	++	++	++	++	15
2	++	o	++	++	++	++	++	10
3	++	++	++	++	++	o	++	15
4	++	++	o	++	++	++	++	15
5	+++	++	++	++	++	++	+++	30
6	++	o	o	++	++	o	+++	15
7	++	o	+++	++	++	o	++	18
8	+++	++++	++++	+++	++++	++	++	ca. 250
9	o	+	++	++	++	++	++	21
10	++	++	++	++	+++	+	++	29
11	++	+++	+++	+++	++	o	o	43
12	o	+++	+++	++	+++	o	o	72
13	o	++	++	o	o	o	o	5
14	++	++	+++	+++	o	o	o	10
15	+	++	o	+	o	o	o	5
16	o	+++	+++	o	++	o	+	36
17	o	++	++	o	o	o	o	4
18	o	+++	+++	o	+		+	26

o = Absent or not seen; + = very infrequent; ++ = infrequent; +++ = frequent; ++++ = very frequent.

or even no eosinophilic leukocytes. In all cases but one (to be described later), granulomas, in various stages of development up to scar formation, were rare. Case 7, however, presented numerous scars in the parenchyma. Case 6 showed no granulomas or scars, but only calcified ova (Table III). Schistosomal granulomata were found in the following sites: arterial intima and lumen, arterial adventitia, periarterial tissues (Figs. 3 to 6, 8 and 9), alveolar septa, and bronchiolar adventitia. When in the intima and lumen, a rare finding (case 1), the

† All heart cavities were dilated due to diffuse chronic myocarditis.

granuloma distended the wall, destroying the media in areas and fragmenting the elastic membranes. The lumen was reduced or occasionally occluded; concomitant hyaline thrombi contributed to the occlusion (Fig. 5). Scars of granulomas were found in the parenchyma, with or without remnants of ova. The scars were irregular, formed by loose or dense connective tissue with round cell infiltration and sometimes contained carbon particles. Occasionally (cases 7 and 10) these scars included one or more wide blood vessels of venular structure, as shown by walls constituted only by intima (endothelium) and adventitia (thin collagenous layer) with thin and inconstant elastic fibers.

Occasional calcified or non-calcified ova, without inflammatory reaction around them, were found in precapillary lumina, alveolar septa, bronchial adventitia, or free in alveoli (Fig. 7).

In passing through the arterial wall the ovum caused focal or diffuse necrosis. Focal necrosis involved either all the arterial layers with pseudo-aneurysm formation, or these layers plus the adjacent tissues, mainly the walls of veins (arteriovenous aneurysms). Recent necrosis was found only in cases 1 and 3, later stages and healed lesions being more frequent (Figs. 3, 4, 6, and 8 to 17). The necrotic areas consisted of granular, highly acidophilic substance, with cellular débris and filamentous fibrin; in this area, all layers of the vessel had been destroyed, including the elastic membranes and collagenous fibers of the adventitia (Figs. 3 and 4). The necrotic area was covered by fibrin, but there was no occluding thrombosis. The intima spared by the necrotic process showed edema (empty spaces), slight or absent lymphocytic infiltration, and endothelial cells. The latter were slightly hyperplastic and in process of mobilization in order to cover the necrotic area and to rebuild the arterial lumen. As a later stage, thrombotic hyaline substance with the same morphologic aspect and staining reactions as those of the hyaline thrombi which formed in vascular lumina, appeared in the destroyed area, mainly in the adventitia and adjacent tissues (Figs. 9 and 10). These thrombi will be described in detail in a subsequent paper.

In the lumen and in the wall, the thrombotic substance became organized and showed blood vessel formation and canalization. The new vessels presented a narrow lumen and cubical endothelium which later became wide and flat. Occasionally there were remnants of the hyaline substance among young vessels. The new vessels which formed in the destroyed area of the arterial wall established communications between the affected artery and the adjacent venous capillaries or pulmonary venules. These intercommunications, which may be considered arterio-

venous aneurysms, were in different stages of development (Figs. 3, 9, and 10 to 14). They were rare (more numerous in case 6) but present in all cases. Sometimes no continuity between the new parietal vessels and the adjacent ones was detected, probably due to the plane of the sections.

Pseudo-aneurysms were found infrequently in 7 cases. They appeared as saccular spaces in communication with the arterial lumen, occupying the destroyed area of the adventitia and the surrounding periarterial tissue (Fig. 6). The sacs were formed mostly by the latter. They were empty, or, less frequently, contained hyaline thrombi in organization. Occasionally transitional pictures occurred between pseudo-aneurysms and arteriovenous aneurysms in which a saccular structure was interposed between artery and vein (Fig. 17). The ova responsible for the formation of pseudo-aneurysms and arteriovenous aneurysms was not found constantly, even in early stages.

In arterial walls focal scars without blood vessels were rare; generally the destroyed area was occupied by wide new vessels.

Old diffuse destruction of arterial walls (mainly of small arteries) was infrequent, except in case 10 (Fig. 16); when present, however, there were still short segments of the wall provided with media, or fragments of the elastic membranes with an adventitial area. In case 7, arterial remnants were occasionally enclosed in the parenchymatous scars. A few arteries with old focal or diffuse destruction of their walls showed either recanalization with an angiomatoid aspect (Shaw and Ghareeb[7]), or that resembling a division of the primitive lumen by fibrous tracts, provided with occasional elastic fibers (Figs. 14 and 15). Occasional structures seen in case 10 must have taken origin in arteries diffusely destroyed, without leaving remnants (Fig. 18). Figure 18 shows, close to a respiratory bronchiole, numerous wide blood vessels, densely packed, in nodular arrangement, with one partially calcified ovum included.

In case 8 the number of lesions due to ova was unusually high, with granulomata in stages of evolution up to scar formation, frequently with more than one ovum or remnants thereof in each lesion (Figs. 19 to 23). The granulomas were found in all localizations described for the other cases, and also in the pulmonary alveoli; in arteriolar and precapillary lumina, however, they were absent. The occlusion of the lumina of small arteries by granulomas was very conspicuous and was seen in all branches of some arteries sectioned longitudinally (Fig. 19). These granulomas developed in the vascular lumina, distending and destroying the arterial wall (circumscribed nodose panvasculitis,

Jaffé,[11] 1948). In a few vessels, remnants of the original lumen were recognizable and permeated by granulation tissue. Some intravascular granulomas showed a necrotic center. Later, new vessels were formed in the granulomatous tissue and the latter became fibrous, with concentric collagenous fibers in the periphery, replacing the media and adventitia of the original vessel. Of such arterial scars, the majority were permeated by wide vessels with venular structure (angiomatoid structure, Shaw and Ghareeb[7]) (Figs. 21 to 23). A few arteries showed a longitudinal series of nodular scars, either isolated or fused to form long fibrous tracts (Fig. 21), as had been seen by gross examination. Many granulomas or, less frequently, scars in a parenchymatous localization had originated in small arteries which were largely destroyed, leaving as remnants only fragments of elastic membranes. Case 8 showed few pseudo-aneurysms and arteriovenous aneurysms. Infrequently, a small amount of fibrin with occasional erythrocytes was found, associated with occluding granulomas or covering areas of the arterial wall destroyed by the formation of granulomas in the adventitia.

For each case the number of lesions due to ova (granulomas and their scars, destructive processes of arterial walls) was about the same in the sections of the various blocks for that case. Lesions due to worms were not found.

Vascular Lesions Unrelated (at Least Directly) to Schistosome Ova

The changes found in small branches of the pulmonary artery and not directly related to ova will be described in a subsequent paper. They are: hyaline thrombi, relapsing endarteritis, and intimal fibrous thickening.

Heart, Liver. Myocarditis was absent in both affected and control groups, except for cases 8 and 12. The former presented schistosomal myocarditis with some granulomas, and the latter a chronic diffuse myocarditis of undetermined etiology. The liver showed, in all cases, the picture of schistosomal cirrhosis; in case 13 Laennec's cirrhosis also was present.

Control Group (Cases 11 to 18)

In all of the control cases of schistosomiasis, the patients were without hypertrophy and dilatation of the right ventricle. As compared to the preceding group, excluding case 8, the frequency of schistosomal granulomas and their scars in the lungs was about the same as in 4 cases (nos. 13, 14, 15, and 17) of the cor pulmonale group and higher

in 4 others (nos. 11, 12, 16, and 18). Granulomas and scars were generally in the so-called parenchymatous location. Cases with heavier parasitization showed, however, that a few parenchymatous lesions inclosed remnants of arteries as well as precapillaries or arterioles, with diffuse or focal destruction of the wall. These findings were frequent in cases 11 and 12, in which infestation was highest. Only in case 12 were granulomas found rarely in the lumina of small pulmonary arteries or in a para-arterial location. In this case the number of active or healed granulomas was somewhat comparable to that of case 8 (of the group with cor pulmonale); occasional conversion of pulmonary arteries into fibrous tracts was found. In no control case were pseudoaneurysms, arteriovenous aneurysms, angiomatoid structures,[7] or hyaline thrombi found, except rare arteriovenous fistulas in cases 16 and 18, and two arterioles in case 12 and one precapillary in case 14 which showed angiomatoid structure.

Discussion

In this necropsy material of schistosomiasis, the incidence of schistosomal cor pulmonale was higher (5.5 per cent) than that found by Shaw and Ghareeb[7] (2.1 per cent) or by Koppisch[2] and Gelfand[27] (0.0 per cent). The frequency would be higher (6.6 per cent) were it not for the exclusion of 2 cases of right heart hypertrophy and dilatation, because material for microscopic study was not available. (See later comment on case 10.) Age incidence (mostly in young persons) is in accord with the data found in the literature.[14] High incidence in females (90.0 per cent) contrasted, however, with previously reported data.[7,14] Our 10 cases of schistosomal cor pulmonale presented also schistosomal hepatic cirrhosis, confirming Meira's[28] clinical observations that schistosomal cor pulmonale is a complication of hepatic involvement. This is understandable, as emphasized by Shaw and Ghareeb, since the ova of *Schistosoma mansoni* are deposited in the portal venous system; their passage to the cava system, and thus to the lungs, is facilitated by the portal hypertension produced by hepatic cirrhosis.

Grossly, schistosomal nodules in the lungs were found in 2 cases only (nos. 8 and 10). However, when cor pulmonale is found at necropsy to be associated with schistosomal hepatic cirrhosis, it should be considered of schistosomal nature.

Microscopically, in the lungs there were vascular changes caused by direct action of schistosome ova, and other changes that were not related to them, at least, directly, which will be described in a subsequent paper. These latter changes were present in small arterial

branches as hyaline thrombi, relapsing endarteritis, and intimal fibrosis. A spatial relationship to ova either was present, or, more commonly, not evident in serial sections. The ova were found either in the pulmonary arterial tree or after penetration into the interstitial connective tissue, reaching occasionally the alveolar lumina, with the possibility of being found in sputum (Fig. 7). In either case, the ova induced the formation of granulomas or pseudotubercles[2] up to scar formation, or did not elicit inflammatory reaction with calcification. On examination of lung sections, an obvious feature was the rarity of granulomas or their scars, as well as of calcified ova, except in case 8, in which such findings were numerous (Table III). The ova were retained mainly in small pulmonary arteries, causing granulomas in the intima and lumen, or in the adventitia; or they crossed the wall, causing focal or diffuse necrosis (arteriolitis necroticans, Shaw and Ghareeb[7]), as well as necrosis of adjacent vessels (Figs. 3 to 6 and 12 to 17). Parietal thrombi and possibly occlusive thrombi (Fig. 15) were associated with these arterial changes. The thrombi were made up of ordinary fibrin, or of homogeneous fibrin with changed staining reactions (hyaline or fibrinoid thrombi). Hemorrhage was prevented by deposition of this thrombotic substance in the necrotic area (Fig. 9). Pseudo-aneurysms, or arteriovenous aneurysms, appeared as a consequence of the necrotic processes involving the arterial walls only, or the arteries and adjacent pulmonary venules and venous capillaries (Figs. 6 and 10 to 14). Venous capillaries might be of pulmonary or of bronchial origin (Fig. 9), both originating pulmonary veins.[1] The arteriovenous aneurysms were of two types: one, more frequent, without a sac between artery and vein, and the other with a pseudo-aneurysmal sac between those vessels (Figs. 14 and 17). The former could be designated arteriovenous fistulas and the latter arteriovenous aneurysms with a false sac.[29] In both structures, however, the communication between artery and vein was not established directly, but through new vessels formed in the necrotic area, which later became very wide. These aneurysms may have a rôle in diminishing the increased pressure in the pulmonary artery.

Such structures as that seen in Figure 18 must have origin in a pulmonary artery which has been diffusely destroyed, leaving no remnants of its wall, in place of which blood vessels were formed.

In case 8 with a great number of ova, the intravascular arrangement of the schistosomal granulomas gave the arteries the aspect of rosaries (Figs. 19 and 21), the radiologic picture of which was described by Erfan and Deeb.[30]

The angiomatoid structures described by Shaw and Ghareeb[7] take

origin either from healed intra-arterial granulomas (Figs. 21 and 22), or from thrombosed recanalized arteries through the walls of which ova have passed (Fig. 15).

In the control group, without schistosomal cor pulmonale, the schistosome ova were retained in smaller branches of the pulmonary artery (arterioles, precapillaries) than in the group with pulmonary hypertension. This may be due, in this latter group, either to a narrowing of the pulmonary vascular bed by endarteritis, to be reported later (mechanical factor), or to a greater allergic reactivity of the arterial intima to ova (specific factor), retaining them in larger vessels. The retention of the ova in vascular lumina greater than the diameter of the ovum, as has been reported by Shaw and Ghareeb,[7] was seen in this material (Figs. 10 and 11). The rôle played by narrowing of the lumen is undeniable, but that of the specific factor is questionable. It is possible, however, that both factors act in the retention of ova.

Based on the total number of lesions due to ova found in sections of lung, we may say that, in the control group, the number of ova which had arrived in the lungs was about the same (cases 14 and 18), smaller (cases 13, 15, and 17), or higher (cases 11, 12, and 16) than in the cor pulmonale group (Table III). Cases with greater numbers of ova (nos. 8, 11, 12, 16, and 18) showed that at least a few granulomas or scars in a parenchymatous location originate in small pulmonary arterial branches (precapillaries, arterioles, and small arteries), since remnants of the latter were found in them (Fig. 22). Possibly other granulomas in that location, without vascular remnants within, have had vascular origin, as has been admitted by Potenza.[12] (Parenchymatous location is employed in the sense I have defined.)

The anatomical changes caused by schistosome ova in the pulmonary arterial tree constitute only a small obstacle to the pulmonary circulation and thus they have a secondary rôle, if any, in the pathogenesis of pulmonary hypertension. Exception is made of case 8, in which the number of ova arriving in the lungs was unusually high, with conspicuous arterial obstruction by schistosomal granulomas. In this case, only, the latter may be largely responsible for the pulmonary hypertension. Case 8 presented a systemic spread of ova (lungs, heart, thyroid gland, kidney, spleen, lymph nodes). The schistosomal granulomas found in the myocardium were not numerous, seeming to have a secondary rôle in producing the marked hypertrophy of the right ventricle (1.5 cm.).

The pathogenesis of pulmonary hypertension in other cases will be discussed in another report. It is possible that sickle cell anemia took part in producing cor pulmonale in case 7. However, pulmonary

arterial thrombosis, as described in sickle cell anemia,[31] was absent in this case.

Possibly, case 12 presented pulmonary hypertension with pathogenesis like that of case 8, due to the great number of ova. However, case 12 was not included in the group of cases of cor pulmonale because the hypertrophy of the right ventricle was slight (0.5 cm.) and there was chronic myocarditis also, which may have been responsible for hypertrophy and dilatation of the right ventricle. In addition, the intimal reactivity of this case, responsible for allergic pulmonary endarteritis (to be reported) was not increased, as in all cases of the control group.

In the pathogenesis of systemic schistosomal granulomas[32] consideration must be given to the passage of ova from the arterial into the venous pulmonary system since, after causing necrosis of arterial and venous walls, the ova may reach the venous lumina (Figs. 12 and 13).

This study and previous reports[14,17,20] show the fallacy of Tidy's[33] assertion that there is no pulmonary schistosomiasis in Brazil.

Summary and Conclusions

The pulmonary vascular changes due to ova of *Schistosoma mansoni* in 10 cases of schistosomal cor pulmonale are reported. Eight additional cases were studied as controls.

It is confirmed that schistosomal cor pulmonale is a complication of schistosomal hepatic cirrhosis. Its incidence was much higher in women than in men (9:1).

The incidence of schistosomal cor pulmonale in 180 necropsy cases of schistosomiasis was 5.5 per cent; considering in this material only the 89 cases in the stage of hepatic cirrhosis, the incidence of schistosomal cor pulmonale is elevated to 11.2 per cent.

Schistosome ova were detained mainly in small pulmonary arteries (100 to 200 μ diameter), causing granulomas in their walls and lumina; or, crossing these walls, destroying them focally or diffusely as well as adjacent vessels. Pseudo-aneurysms and arteriovenous aneurysms were formed. The latter may have a rôle in diminishing the increased pulmonary arterial pressure. Non-occlusive fibrin thrombi were associated with these lesions due to ova.

The passage of ova from pulmonary arteries into pulmonary veins through necrosis of their walls must be accepted in schistosomiasis. This is of significance in the pathogenesis of systemic distribution of schistosomal granulomas.

The anatomical changes caused by schistosome ova in the pulmonary arterial tree had a secondary rôle, if any, in the pathogenesis of

pulmonary hypertension, except in one case (no. 8) in which arterial obstruction was due mainly to schistosomal granulomas.

In the control group without cor pulmonale, the ova were retained in smaller branches of the pulmonary artery (vessels below 100 μ diameter), possibly due to absence of an increased allergic intimal reactivity and a diminution of the pulmonary arterial bed caused by endarteritis (to be reported later).

Schistosomal granulomas with a parenchymatous location may originate in pulmonary arterioles, precapillaries, or, less frequently, in small arteries.

I am indebted to Dr. Alvaro de Freitas Armbrust for helping with the statistic data, and to Dr. Orlando Aidar for reviewing the English text.

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LEGENDS FOR FIGURES

FIG. 1. Case 1. Heart. Normal aspect of the left ventricle.

FIG. 2. Case 1. Heart. Marked hypertrophy and dilatation of the right ventricle.

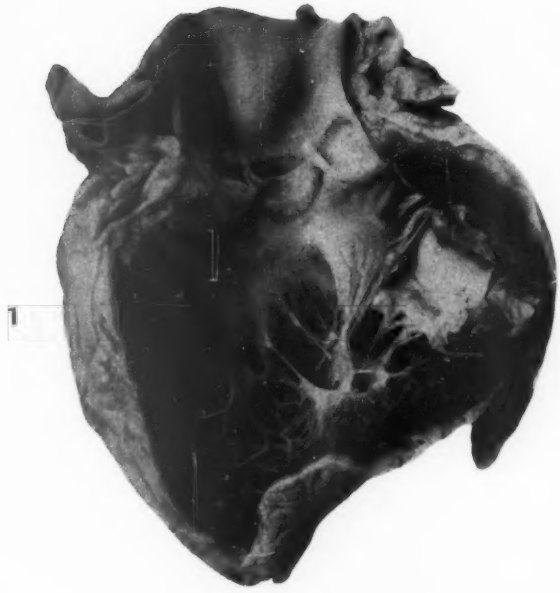

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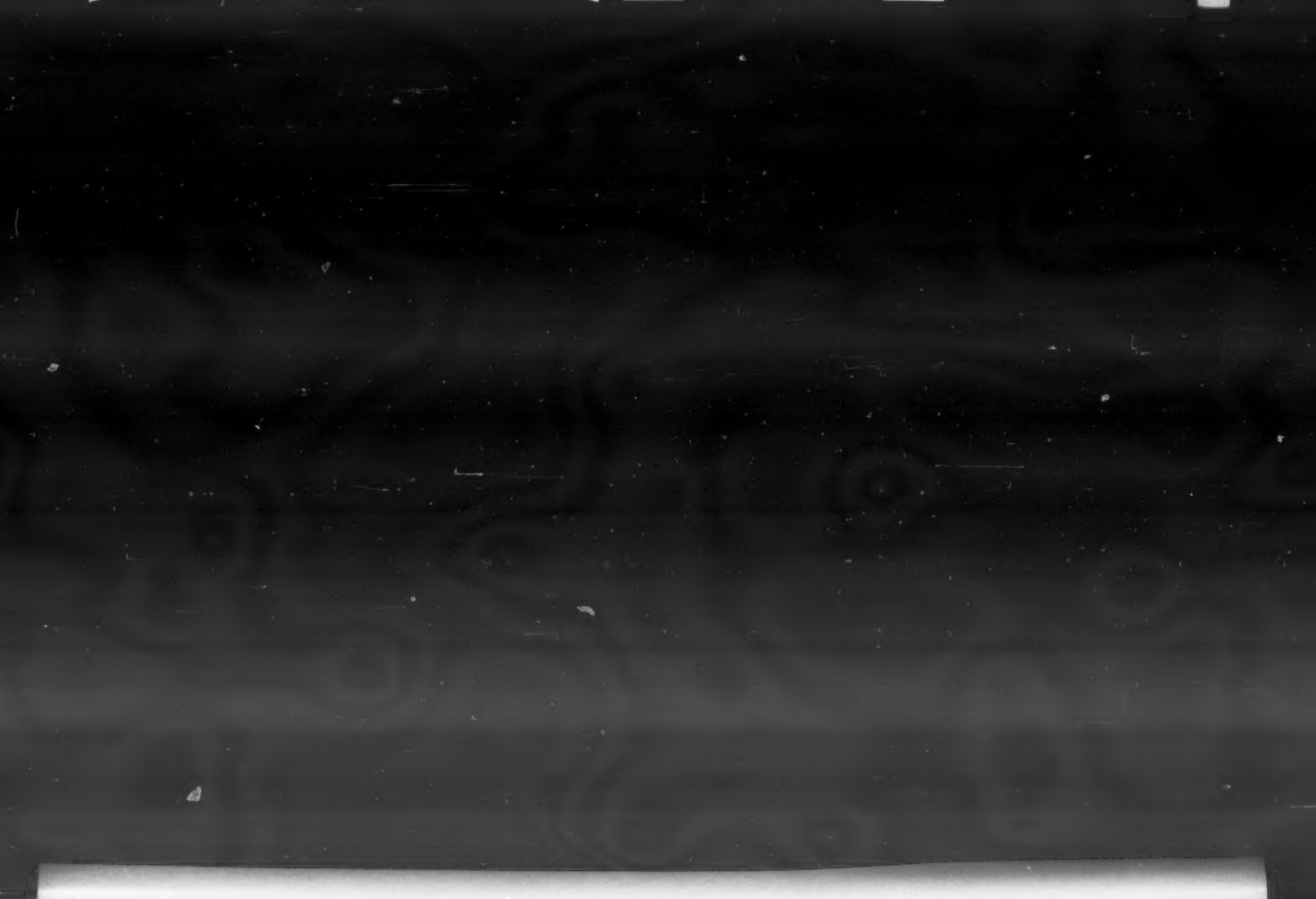
FIG. 3. Case 1. Small pulmonary artery, 150 μ in diameter. Focal necrosis of the arterial wall and of adjacent venule (right of arrow) near ovum. Initial stage of arteriovenous aneurysm. The ovum is covered by a non-occlusive thrombus. Early granulomatous reaction in the arterial adventitia adjacent to the necrotic area. At the necrotic area of the venule, a hyaline thrombus (arrow) is laid down, marginated by histiocyte-like cells. Hematoxylin and eosin stain. × 306.

FIG. 4. The same artery as shown in Figure 3. Its upper left part shows partial loss of both elastic membranes, especially the internal. The preserved intima (arrow) shows a few clear spaces (serous endarteritis). Weigert's elastic tissue and van Gieson's stains. × 384.

FIG. 5. Case 1. Schistosomal granuloma within arterial lumen associated with hyaline thrombus (at left) with dilatation of the lumen. At left, in the lower part, the intima shows intense thickening with narrowing of the lumen (x). The arterial wall is replaced by cellular infiltration in the right lower quadrant; here the internal elastic membrane is indicated by the arrow. Weigert's elastic tissue and van Gieson's stains. × 330.

FIG. 6. Case 4. Small pulmonary artery with a pseudo-aneurysm, the sac (x) of which does not communicate, in this section, with the highly narrowed arterial lumen. A giant cell in the adventitia surrounds the ovum (arrow) responsible for arterial necrosis. Weigert's elastic tissue and van Gieson's stains. × 100.

FIG. 7. Case 1. Schistosome ovum free in an alveolar lumen and marginated by macrophages loaded with pigment. Weigert's elastic tissue and van Gieson's stains. × 224.



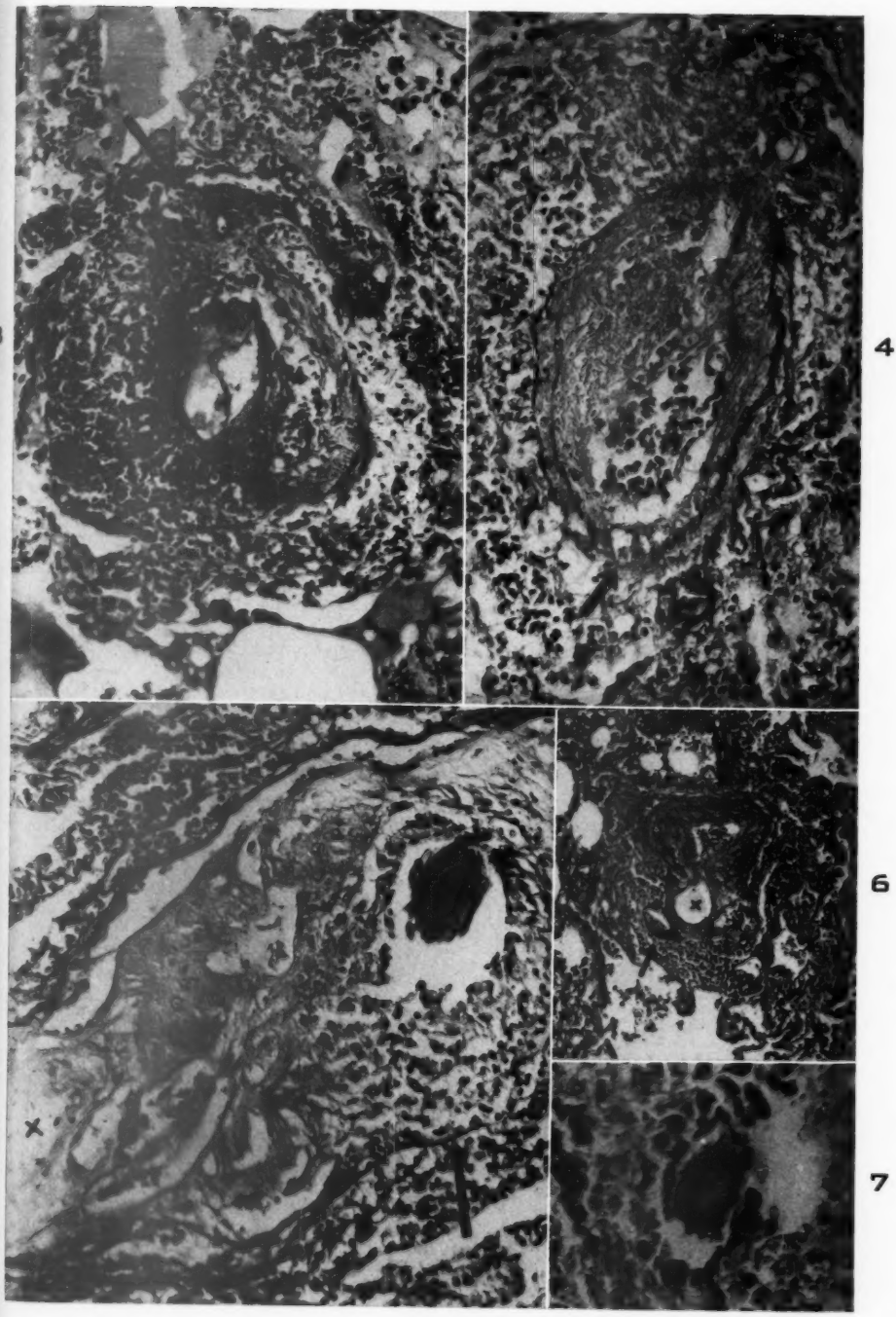

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FIG. 8. Case 1. Focal destruction of the wall of a small pulmonary artery by a schistosome ovum, which appears as a black spot at the left upper quadrant. In the destroyed area, the arterial lumen is occluded. There is deposition of thrombotic hyaline substance between this area and the granuloma formed by the ovum (arrow). Upper arrow shows an arteriole with almost occluded lumen. Weigert's elastic tissue and van Gieson's stains. × 100.

FIG. 9. Same artery as shown in Figure 8, in a subsequent section. Above the artery there appears the schistosomal granuloma without the ovum. The non-occluded part of the arterial lumen is seen in the left lower quadrant (x). Fibrinoid hyaline substance, seen mainly in the periarterial tissue, is in organization; a vessel (clear cleft) is being formed in the middle of this substance. This vessel crosses the arterial wall and opens into a venous capillary, 30 μ in diameter (to the right). This illustrates an arteriovenous fistula arising through organization. Hematoxylin and eosin stain. × 300.

FIG. 10. Case 1. Larger pulmonary artery (lumen, 172 μ in diameter) showing focal destruction of the wall. The ovum responsible for the necrosis does not appear in this section. In the destroyed area of the adventitia, there are a remnant of thrombotic hyaline substance (left arrow) and new capillaries (clear spaces), which established communications, as seen in serial sections, between the artery and an adjacent dilated venule (right arrow). Arteriovenous fistula formed by organization. Hematoxylin and eosin stain. × 100.

FIG. 11. Same artery as shown in Figure 10 presenting, besides intimal fibrous thickening, a newly formed tissue at the destroyed area, with capillaries in various stages of development. These capillaries appear as wide clear spaces (x), as cellular agglomerates with narrow lumina (lower arrow), and as hyperemic capillaries (upper arrow). The adjacent wall of the venule is formed only by this proliferated tissue. Weigert's elastic tissue and van Gieson's stains. × 300.

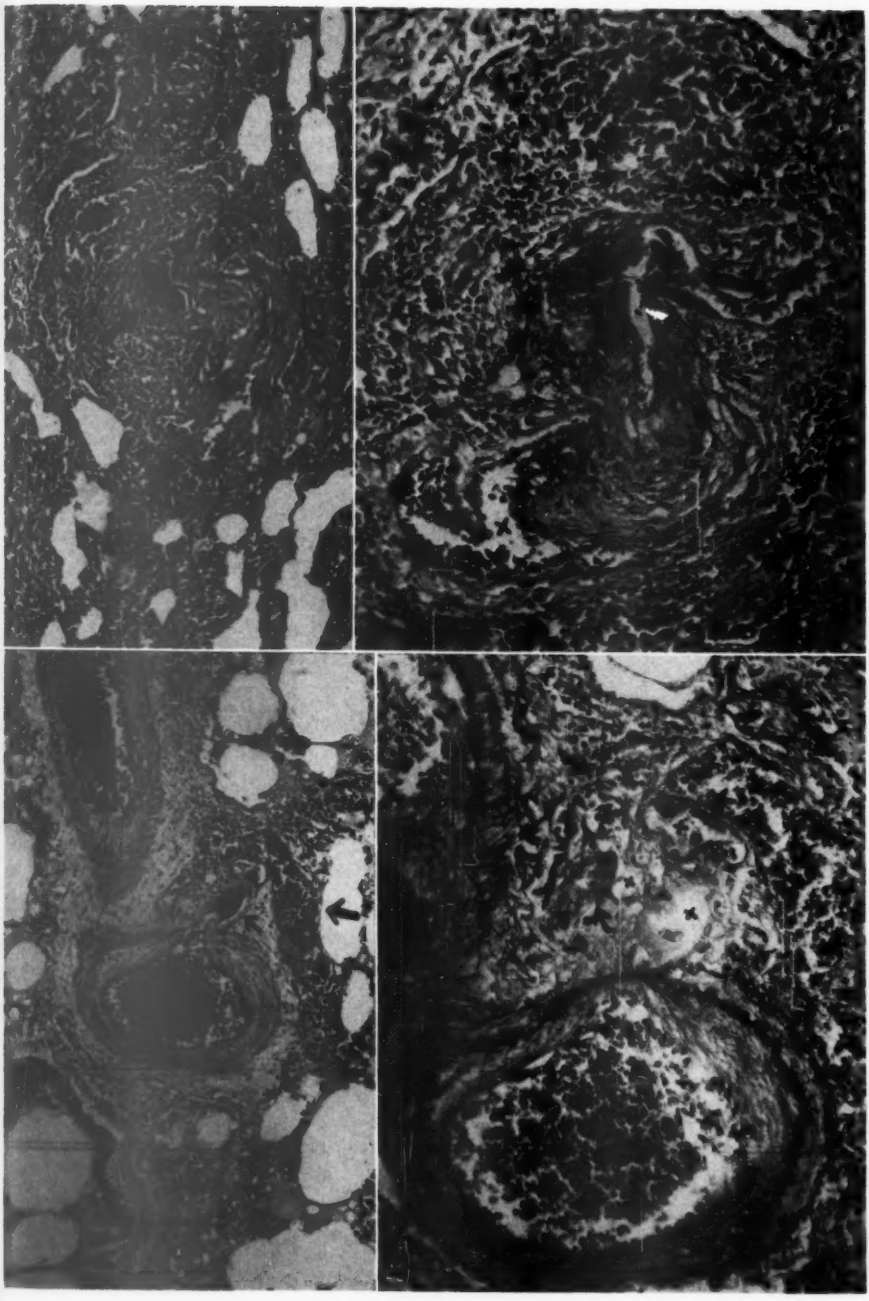


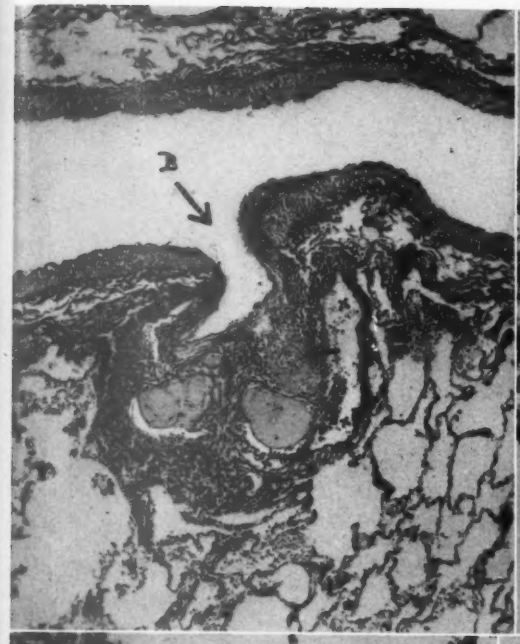
FIG. 12. Case 3. A schistosome ovum (arrow) crossed the wall of an arterial branch (arrow B), causing necrosis which also involved the wall of an adjacent small venule, 99 μ in diameter (x). Hyaline thrombi are associated with the necrotic process. One of these at the right is bulging into the venous lumen (x). Weigert's elastic tissue and van Gieson's stains. × 80.

FIG. 13. Higher magnification of Figure 12. Necrosis of the arterial and venous walls is conspicuous. The schistosome ovum is surrounded by necrotic tissue mixed with fibrin. In the venous lumen (above) a leukocytic thrombus is superimposed on the hyaline thrombus. × 300.

FIG. 14. Case 1. Small pulmonary artery with recanalization showing a wide communication (arrow) with the adjacent venule at the left (arteriovenous fistula). Weigert's elastic tissue and van Gieson's stains. × 300.

FIG. 15. Case 1. Small pulmonary artery with interruption of the media (passage of ovum), indicated by the arrow, with thrombosis and recanalization of the lumen. Endothelial cells in one of the new vessels are hyperplastic, forming more than one layer. Hematoxylin and eosin stain. × 240.

FIG. 16. Case 4. A small pulmonary artery which has been extensively destroyed. Only a short segment of the wall (left arrow) has been preserved. Wide, newly formed vessels and a hyaline thrombus in organization (lower arrow) are seen in place of the destroyed artery. Weigert's elastic tissue and van Gieson's stains. × 100.



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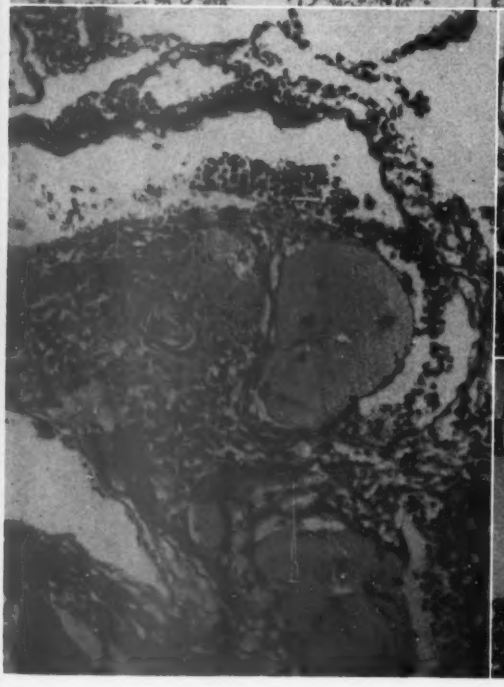

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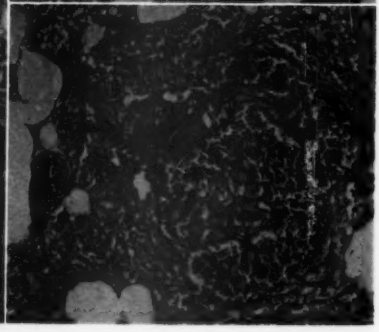

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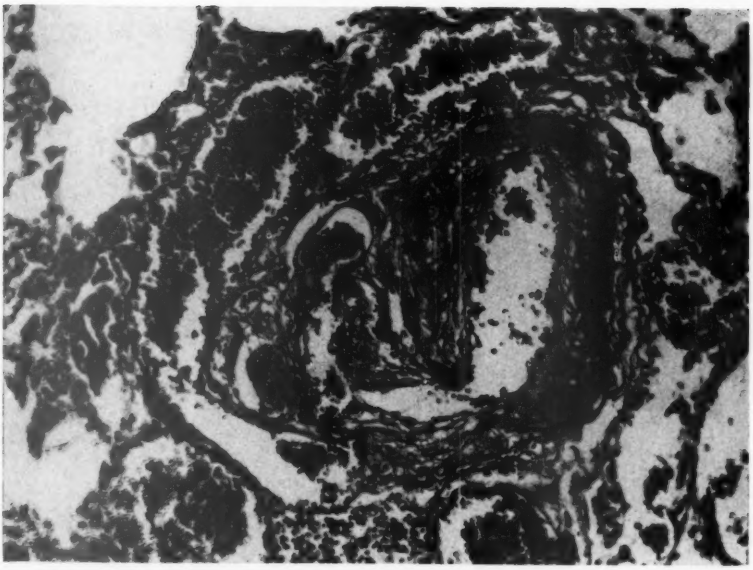
FIG. 17. Case 2. Larger pulmonary artery and a small vein (at its left) forming an arteriovenous aneurysm with a false sac. The sac shows hyaline thrombotic masses in organization, with new vessels. Continuity is not apparent in this section between the lumina of the new vessels and that of the vein, the wall of which here is largely formed by the newly proliferated tissue. Weigert's elastic tissue and van Gieson's stains. × 240.

FIG. 18. Case 10. Wide new blood vessels, in nodular arrangement, with one partially calcified ovum, close to a respiratory bronchiole (above). This angiomatous structure must have taken origin in a diffusely destroyed branch of the pulmonary artery. Weigert's elastic tissue and van Gieson's stains. × 300.

FIG. 19. Case 8. All the branches of a small pulmonary artery (near center) are occluded by schistosomal granulomas (arrows). The conglomerated granuloma (lower right arrow) and also the other granulomas indicated by arrows are of intra-arterial origin. Weigert's elastic tissue and van Gieson's stains. × 22.



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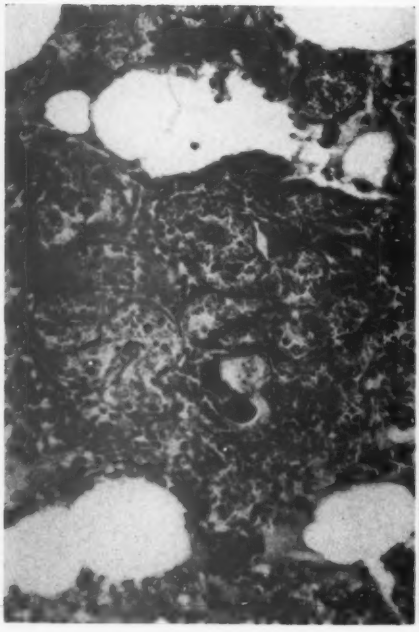

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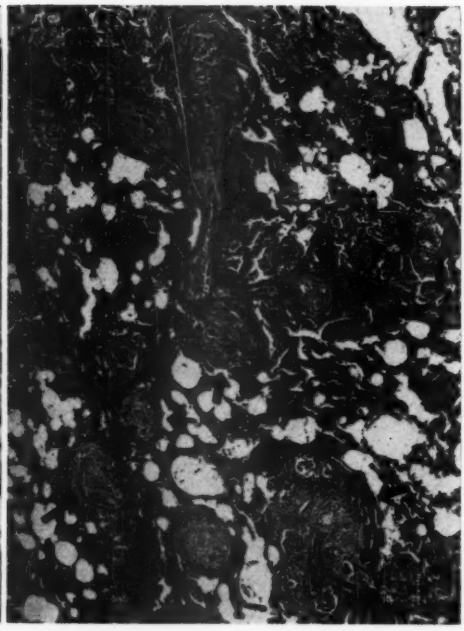

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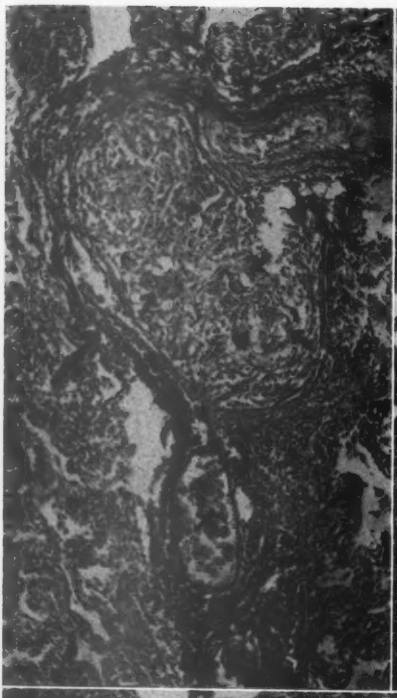
FIG. 20. Case 8. Small pulmonary artery. Occlusion of the lumen by conglomerated schistosomal granulomas, with four ova surrounded by giant cells (small arrows). Dilatation and destruction of the arterial wall, with remnants only of the elastic membranes (large arrow) and adventitia. The upper arterial segment shows strong intimal fibrous thickening with narrowing of the lumen; the lower segment, without intimal fibrosis, presents dilatation of the lumen (x). Weigert's elastic tissue and van Giesons' stains. × 100.

FIG. 21. Case 8. Longitudinal section of a pulmonary artery (in the lower third of the vertical axis of the field) and its two branches, showing their substitution by scar tissue with nodular structures (arrows) and wide vessels. Weigert's elastic tissue and van Gieson's stains. × 22.

FIG. 22. Case 8. Nodular scar which has developed in a pulmonary artery and is now permeated by wide new vessels; of that artery only remnants of the elastic membranes were preserved (arrow). This scar must have taken origin from an intra-arterial granuloma. Weigert's elastic tissue and van Gieson's stains. × 100.

FIG. 23. Case 8. Detail of the arterial scar from Figure 21, indicated by arrow D, probably developed from a schistosomal granuloma. × 300.



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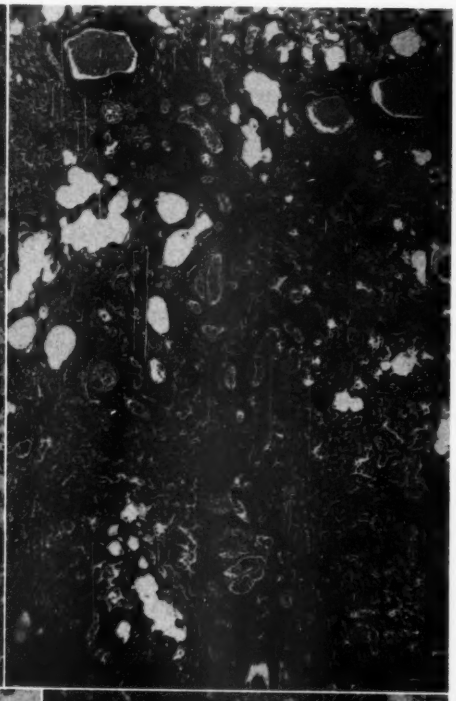

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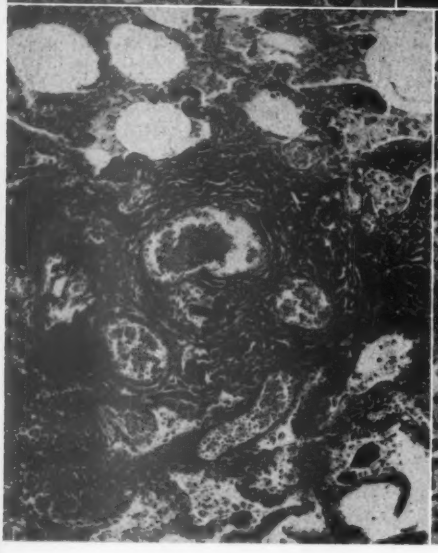

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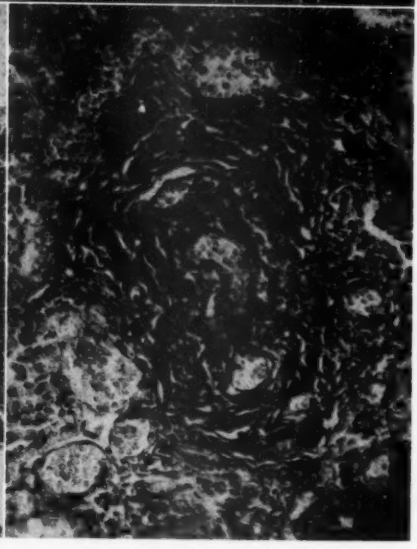

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